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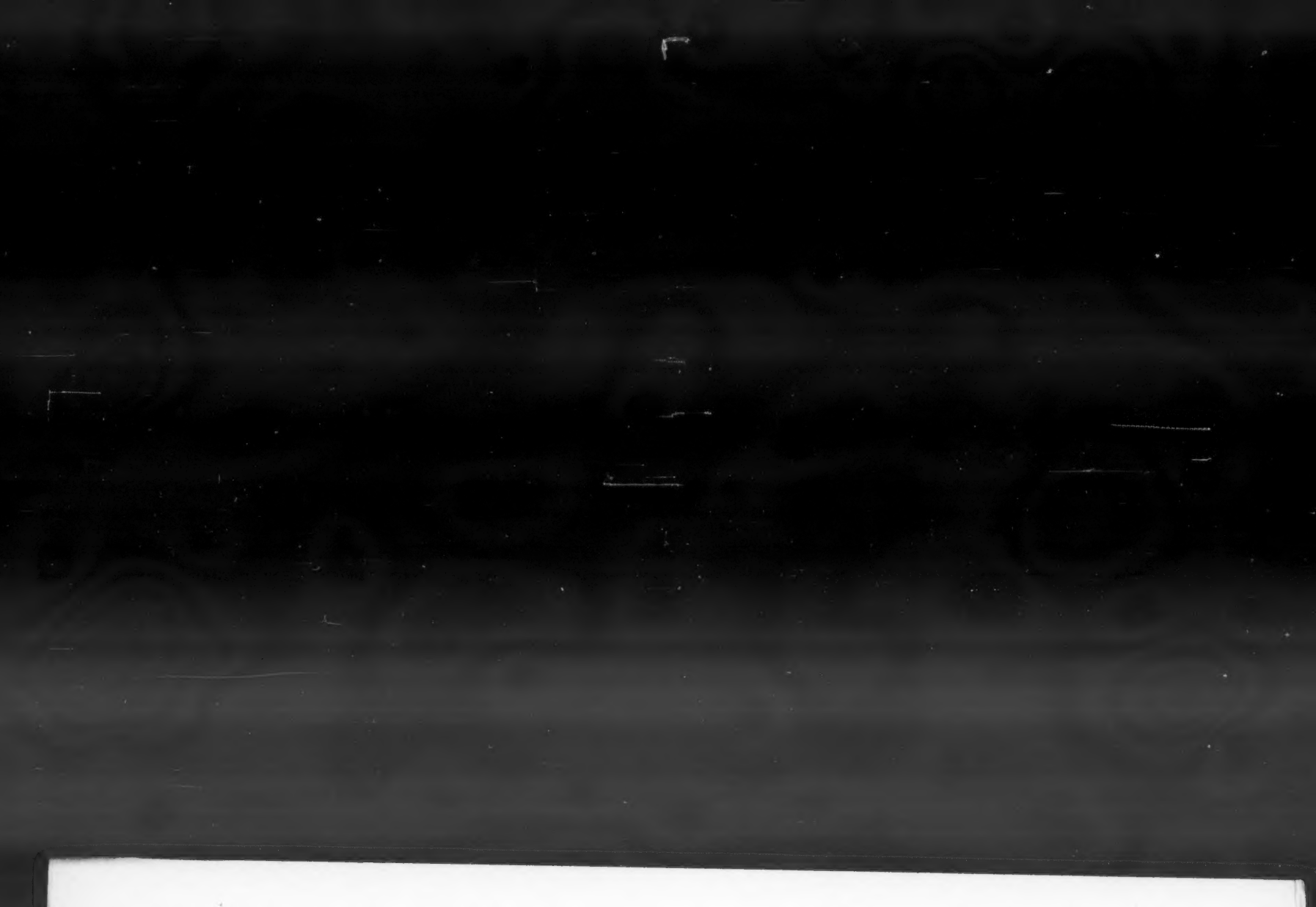

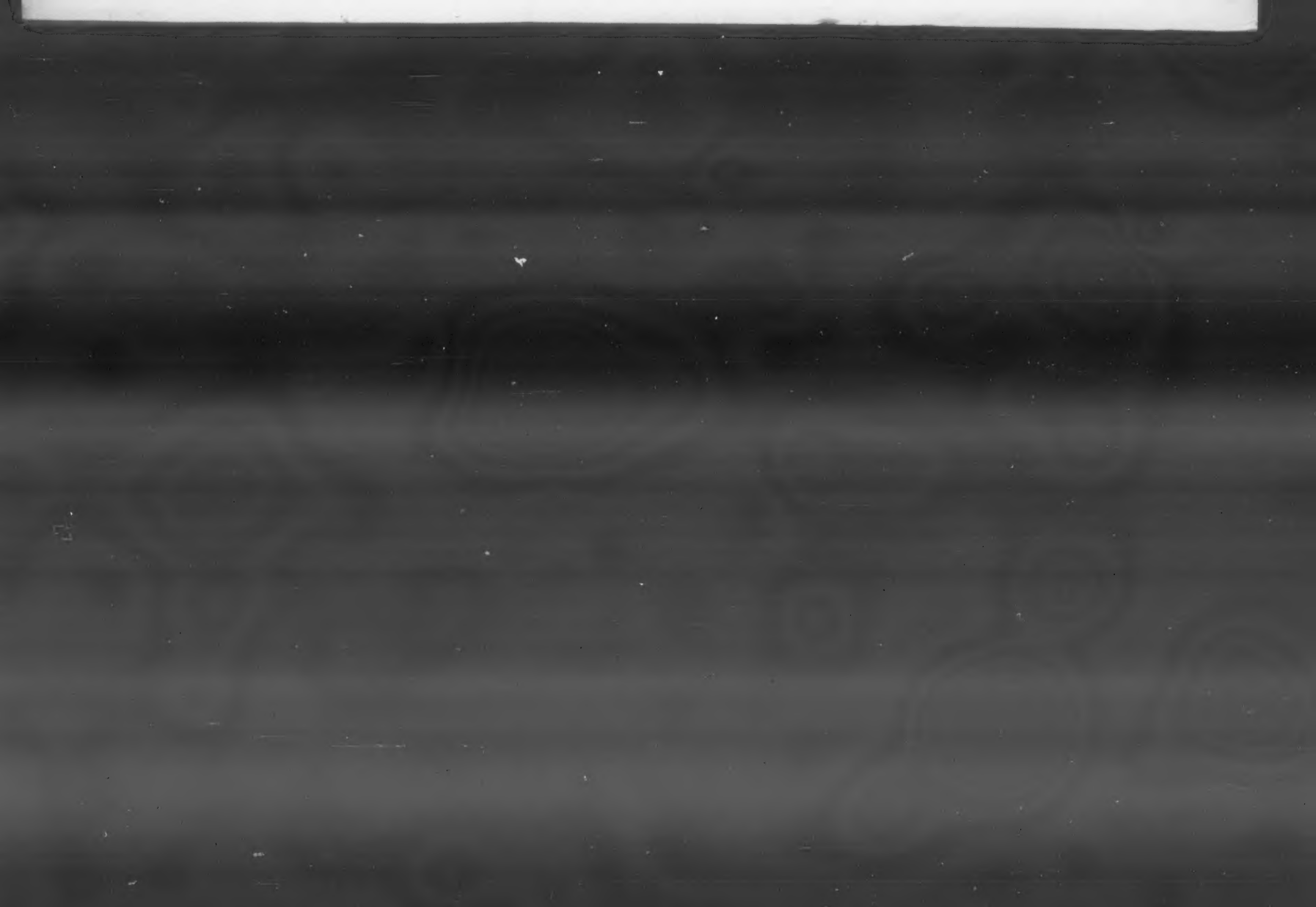

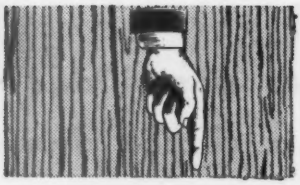

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A FUNCTIONAL DISCONTINUITY BETWEEN THE PURKINJE AND VENTRICULAR MUSCLE CELLS

J. Alanís, Daisy Benítez and G. Pilar (*)

(Department of Physiology, Instituto Nacional de Cardiología, México.)

The experimental results reported in a previous paper (Alanís and Pilar, 1960) suggested the existence of a functional discontinuity between the ventricular muscle fibers and the Purkinje cells. The aim of the present study was to investigate this possibility further by means of the intracellular recording technique, which permits a precise measurement of the temporal relationships of the electrical events of these two cardiac tissues.

Methods

Dogs were used, anaesthetized with sodium pentobarbitone. The hearts were excised and immersed into a bath containing a Tyrode solution at 37° C. The right ventricle was opened and a papillary muscle with its attached false tendon was fixed with glass needles in a small chamber (20 ml) filled with an aereated Tyrode solution. Thin papillary muscles were chosen in order to minimize the injured area of the base of the severed muscle, and also to have a better diffusion.

The composition of the Tyrode solution was the following: NaCl – 137 mM, KCl – 2.7 mM, $CaCl_2$ – 1.8 mM, $MgCl_2.6H_2O$ – 0.5 mM, $NaH_2PO_4.H_2O$ – 0.4 mM, $NaHCO_3$ – 12.0 mM, dextrose 5.5 mM. The gas mixture was 95 per cent O_2 and 5 per cent CO_2. The temperature of the chamber was kept between 36 and 37° C and the flow rate of the solution was maintained at 12 to 20 drops per minute. The chamber was connected to ground through a silver wire.

The stimulation of either the papillary muscle or the Purkinje fibers was performed through fine platinum needles inserted into the tissue and connected to a square pulse generator; one of the electrodes was grounded. In some cases the tissues were stimulated intracellularly with micro-pipettes filled with 3M KCl. The intensity of the stimuli was quantified in threshold units.

The transmembrane action potentials of both the papillary muscle and the Purkinje fibers were recorded with micro-electrodes (glass micro-pipettes filled with 3M KCl made according to the techniques of Ling and Gerard,

(*) Fellow of the "Consejo Nacional de Investigaciones Científicas y Técnicas de la R. Argentina".

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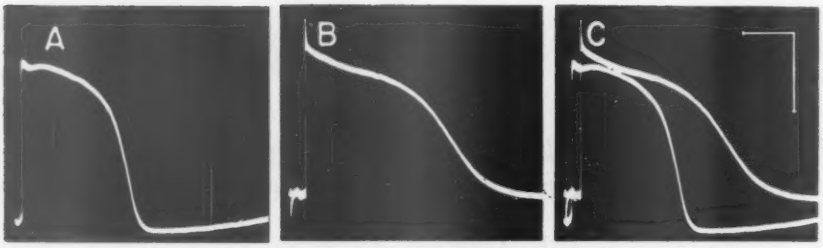

FIG. 1. — *Transmembrane potentials recorded from a ventricular muscle cell (A) and from a Purkinje fiber (B). Note the different shape and duration of the responses and the latency between the activation of the ventricular muscle and the Purkinje fiber (C). Papillary muscle driven at 1.0/sec. Calibrations: 100 msec and 50 mV.*

1949 and Nastuk and Hodgkin, 1950). The recording micro-electrodes were connected to one end of a cathode follower, with a grid current less than 10^{-13} amps. The other end was grounded through the solution of the chamber. The action potentials were amplified by means of d. c. preamplifiers (P6 Grass) and recorded in a double-beam cathode-ray oscilloscope (Tektronix).

RESULTS

Propagation of impulses from the papillary muscle to the Purkinje fibers (M-P) and from the Purkinje tissues to the papillary muscle (P-M)

When two micro-electrodes were introduced, one into a Purkinje and the other into a myocardial cell, the corresponding potentials differed in shape and duration (figs. 1, 3 and 5). The individual characteristics were used as a criterion for identifying the type of cells which generated the action potentials.

Intracellular potentials obtained from two sites, one from a myocardial cell and the other from a Purkinje fiber, showed a latency between the activation of the two structures. The value of this latency depends on several factors. In the experiment illustrated in figure 2, when one end of the papillary muscle was stimulated, the M-P latency was 18 msec (fig. 2 A), but when the false tendon was stimulated, the P-M latency was greater, 32 msec (fig. 2 B). Similar differences were found in all the experiments. Spontaneous impulses originated in the Purkinje fibers activated the papillary muscle with a P-M latency of aproximately the magnitude shown in figure 2.

The values of both latencies were only slightly modified by variations in the distance between the two recording micropipettes. The interelectrodal distance ranged in general between 0.1 to 6.0 mm. In the case of myocardial conduction (M-M) and for the experiment illustrated in figure 2 C, the impulse took only approximately 2.5 msec for travelling 2 mm. The conduction time for the Purkinje tissue (P-P) was of 0.3 to 0.4 msec for travelling a distance of approximately 1.5 to 2.0 mm (fig. 2 D). Adding these two values would give only 2.9 msec, a considerably smaller value than either that of the M-P latency (18 msec) or that of the P-M latency (32 msec).

The average values of the latencies were the following: 18 msec for the M-P, 29 msec for the P-M, 1.3 msec for the P-P and 3.0 msec for the M-M

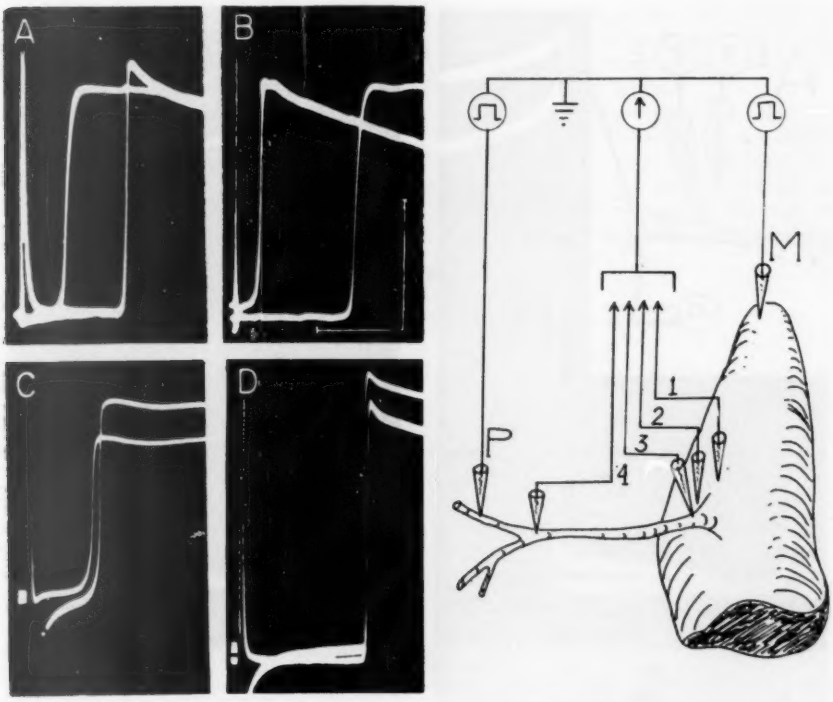

FIG. 2. — *Action potentials recorded from ventricular and Purkinje single fibers. Diagram shows the position of stimulating and recording electrodes on the false tendon and papillary muscle. Micro-electrodes 1, 2, 3 and 4 are for recording, M and P for extracellular stimulation. The myocardium was stimulated in A, C and D. In B the stimuli were applied to the false tendon (1.0/sec). There appeared a latency between the myocardium and Purkinje responses. The latencies M-P (A) and P-M (B) have not the same magnitude. These records were taken with electrodes 2 and 3 which were separated each other 1.5 mm. A and B were taken without moving the micropipettes. In C, two myocardial potentials recorded with micro-pipettes 1 and 2 separated each other by 3 mm. D, papillary muscle stimulation and recordings from two Purkinje cells (micro-electrodes 3 and 4 separated 8 mm). The conduction time of both myocardium plus Purkinje does not explain even the shortest latency (M-P). Calibrations: 30 msec and 50 mV.*

latency (see Table I). The values of the M-P and P-M latencies were recorded even when the cardiac structures were driven at low frequencies (0.5-1.0 per second; figs. 1 and 5).

That the long M-P and P-M latencies do not depend on the interelectrodal distance is illustrated in figure 3. In record A the micro-electrodes were placed on the false tendon and the separation between them was 0.2 mm. The potentials recorded with both electrodes (see diagram) appeared as typical Purkinje-cell electrograms with a brief delay. When electrode 2 was advanced 15 to 30 microns, the potentials which corresponded to this electrode showed the characteristics of a myocardial res-

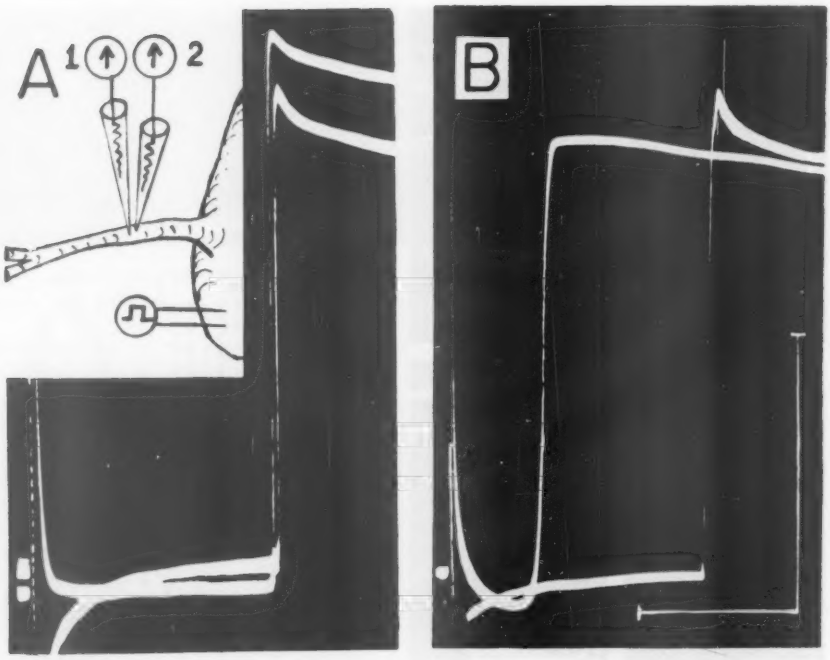

FIG. 3. — *Transmembrane potentials from two Purkinje fibers (A) recorded with electrodes 1 and 2 placed on the false tendon and separated each other by 0.2 mm. Square pulses applied to the papillary muscle (1.0/sec). When the micro-pipette 2 was advanced downward only 15μ, the electrogram recorded with it, suddenly changed its characteristics and appeared preceding the Purkinje potential for several msec (B). In this case for an extremely short distance the M-P delay is considerable (21 msec).*

ponse and the latency between the M and P records became suddenly much longer (fig. 3 B).

Occasionally it was possible to register with a single micro-pipette an intracellular potential, e.g Purkinje cell electrogram, and simultaneously a small extracellular potential which appeared several milliseconds later, originated in a myocardial cell, and therefore, presenting a P-M latency. A downward displacement of the micro-pipette of approximately 5 to 25μ, changed suddenly the myocardial response in its amplitude and shape. The new potential showed the typical characteristics of an intracellular myocardial potential and occurred 20 msec later than the Purkinje potential which became small and diphasic (extracellular).

In some experiments the propagation from myocardium to Purkinje fiber was blocked and in others the block occurred in the opposite direction. In each case direct stimulation of the distal tissue was able to evoke the corresponding response.

Factors which modify the M-P and the P-M latencies

a) *Frequency of stimulation.* When a

papillary muscle was activated directly with progressively increasing frequencies, the latency between the initiation of the M and P potentials augmented gradually until the P potential disappeared at a frequency of 5.7 per sec, as an average (figs. 5 and 8 A, B), while the ventricular fibers could follow frequencies up to 8.5 per sec, as an average. The lengthening of the M-P latency is illustrated in curve B of figure 8.

When the same procedure was followed, applying the stimuli to the false tendon, the P-M latency also increased until the M potential failed (fig. 8 C, D). This block occurred at an average frequency of 4.7 per sec, although the Purkinje-fiber response followed frequencies up to 7.3 per sec.

Variations of the frequency produced also modifications in the amplitude and duration of the M and P potentials. Both the amplitude and the duration of these potentials decreased as the frequency increased (fig. 8 A, C).

b) *Asphyxia.* Brief periods of asphyxia (2-3 min), produced by discontinuing the aereation of the Tyrode solution, elicited a lengthening of the M-P and P-M latencies (fig. 6). For the M-P, the lengthening reached approximately 50 per cent of the initial value in 12 min. The P-M propagation could be blocked if the period of asphyxia was longer than 5 min (fig. 6 C, D). When the periods of asphyxia lasted more than 8 min, the changes produced were irreversible.

c) *Effect of the diminution of NaCl.* When 50 per cent of the NaCl in the solution was replaced by dextrose (177 g per liter), both the M-P and the P-M latencies increased (fig. 7). This increase appeared after 5 min of perfusion with the modified solution. The M-P latency reached its maximal lengthening (150 g per liter), both the M-P and the P-M latency lengthened progressively until the M potential disappeared. The P-M propagation was always blocked before the propagation in the M-P direction. When the solution contained no NaCl, the same phenomenon occurred, but within a shorter period.

d) *Action of quinidine.* In some observations quinidine sulphate was added to the perfusion chamber (100-200 μg). After 2 minutes the M-P and P-M latencies increased gradually during several minutes (6-8) until they attained steady values (fig. 9).

Discussion

The excised false tendon attached to a papillary muscle is a preparation

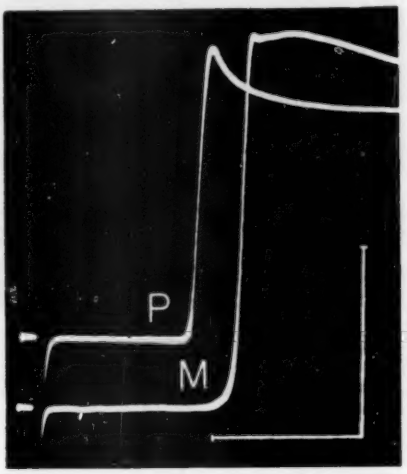

Fig. 4. — *Latencies of ventricular fiber (lower trace) and of a Purkinje cell (upper trace) transmembrane potentials. The records were taken separated and the pictures were superimposed. When a ventricular muscle cell was stimulated intracellularly, the corresponding Purkinje electrogram (P) preceded by several msec (8.5) the ventricular response (M) provoked when the stimuli (1/sec) were applied through the intracellular electrode, to the Purkinje cell. The latency between these two records is the difference in magnitude of the M-P and P-M latencies. Calibrations: 30 msec and 50 mV.*

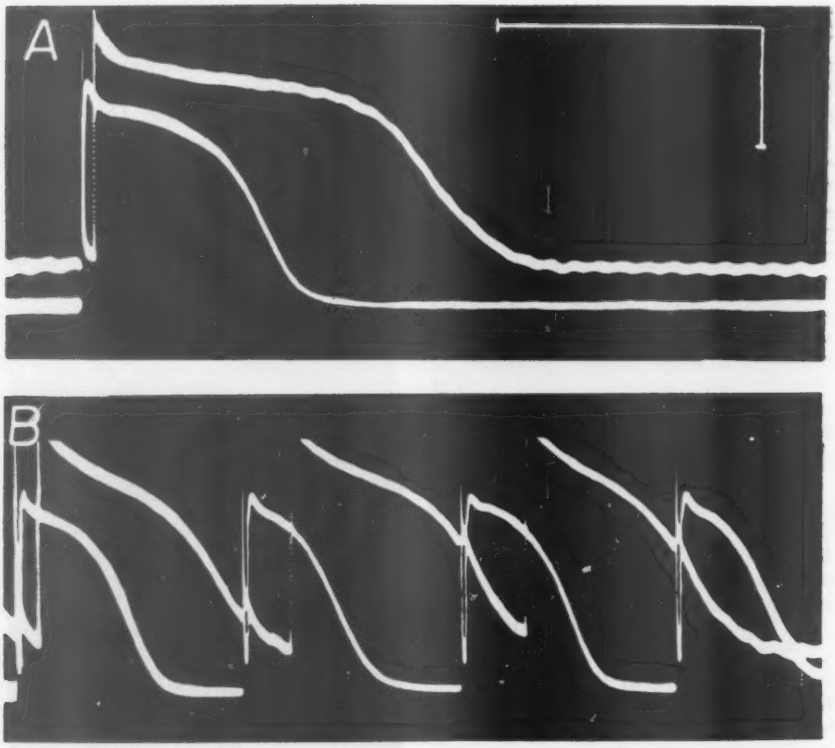

FIG. 5. — *Effect of progressively increasing of frequency on the magnitude of the M-P latency. A Papillary muscle stimulated at 0.5/sec. The myocardial potential appeared 20 msec before than the Purkinje cell electrogram. B. During the high frequency stimulation period (4.0/sec). Note the progressive lengthening of the M-P latency and finally the disappearance of the Purkinje potential. It is evident also the diminution of the duration of both Purkinje and myocardium transmembrane potentials. Calibrations: 300 msec and 40 mV.*

which does not have constant physiological properties for long periods of time. Because of the experimental limitations imposed by the perfusion technique, the recordings were always obtained during the first hour of the experiments, since afterwards the tissues become edematous. In spite of the gradual deterioration of the cardiac fibers, the consistency of the present results and their similarity to those obtained in isolated hearts perfused with blood (Alanís and Pilar, 1960), make the preparation reliable.

Both the papillary muscle and the false tendon contain Purkinje and ventricular muscle cells. The typical configurations and different durations of both electrograms, however, make it easy to identify the type of cell which generates a given potential. These characteristics have been described in previous publications (Draper and Weidman, 1951; Trautwein and Zink, 1952;

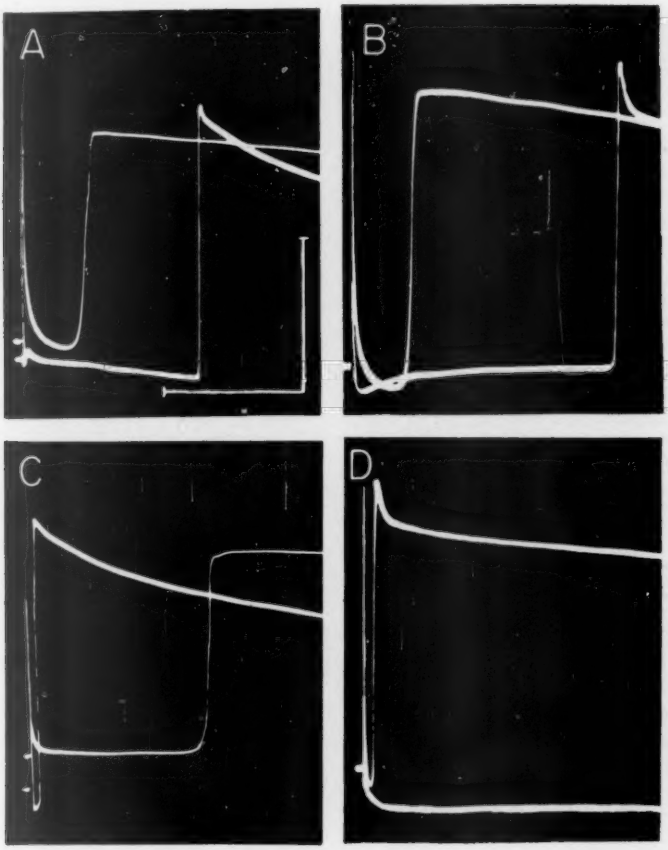

FIG. 6. — *Effect of a short period of asphyxia on the M-P and P-M latencies. A. Ventricular and Purkinje potentials when the myocardium was stimulated. C. The same electrograms when the Purkinje fibers of the false tendon were stimulated. B. and D. after 9 minutes of asphyxia. Note the lenthening of M-P latency and the irreversible blockage of P-M propagation, which is evident by the disappearance of the ventricular potential in C. Calibration: 40 msec and 50 mV.*

Coraboeuf, Distel and Boistel, 1953; West, 155 a, b; Cranefield and Hoffman, 1958). Selective stimulation of one or the other tissue can be attained because of the differences in their excitability or by stimulating intracellularly fibers previously identified by their potentials.

The M-P and P-M latencies

The large magnitude of both the M-P and the P-M latencies (figs. 1, 2, 5, 6, 7) cannot be explained by the time taken by the impulses to travel along the two tissues involved. The values for M-M plus P-P conduction times are

considerably smaller than the values of the M-P or P-M latencies (see Table I). These long latencies occur even when the distance between an electrode in a ventricular muscle cell and another in a Purkinje fiber, is quite small (15-30 μ; fig. 3). When the electrodes were both placed in either muscle or Purkinje fibers with larger distances (2-6 mm), the delays were much shorter.

Long M-P and P-M latencies were found at all the frequencies of stimulation employed (0.5 to 5.6 per sec). These observations do not agree with the results reported by Hoffman, Kao and Suckling (1957), who recorded long latencies only when stimulating at high frequencies. This discrepancy may be due to the fact that these authors used a sweep speed which does not permit an accurate measurement of the latencies in question.

In some experiments it was possible to record with a single micro-pipette the M-P or P-M latencies. In these cases one of the electrode was intracellular for one of the elements and extracellular for the other one. When the depth of the electrode was changed slightly (e.g. 20 μ) the extracellular record could change to an intracellular one, and vice versa, without any modification in their temporal relationships. This can be explained if one supposes that an unsealed hole was produced in the impaled cell membrane, which allowed the recording of the electrical activity of a neighboring cell. This cell was probably a ventricular muscle fiber, since a small downward displacement of the micro-electrode was sufficient to record a typical intracellular papillary-muscle electrogram.

One might attribute the long delays to a deficient perfusion of the fibers. The geometry of the false tendon, however, permits an adequate diffusion of ions and oxygen across the cell membranes (Draper and Weidman, 1951; Weidman, 1952), and the long latencies appear in the false tendons either connected or disconnected from the papillary muscle.

The polarization of the Purkinje-ventricular muscle fiber junctions

The present results indicate that the impulses that travel from Purkinje to ventricular fibers, or vice versa, are delayed at the junctions of the two elements. The following observations

TABLE I

M-P	*P-M*	△	*P-P*	*M-M*
32.5	48.0	15.5	2.7	1.3
16.0	37.0	21.0	1.3	3.4
12.5	30.0	17.5	2.7	1.8
15.6	21.0	5.4	2.7	2.3
16.2	22.0	5.8	1.2	5.2
13.0	27.3	14.3	2.0	5.2
23.8	34.5	10.7	2.6	2.9
27.6	38.0	10.4	0.5	1.0
27.0	36.5	9.5	0.5	4.1
17.4	20.0	2.6	0.5	1.0
15.2	31.5	16.3	1.0	5.1
13.2	28.0	14.8	0.2	
13.2	28.0	14.8	0.1	
11.2	13.3	2.1	0.3	
19.6	21.3	1.7		
14.4	27.4	13.0		
	AVERAGES			
18.0	29.0	11.0	1.3	3.0

Values of the M-P and P-M latencies for 16 experiments. For each case the former latency is smaller than the latter. The difference between them is in the column △. *Note that the conduction time for either P-P or M-M is extremely low compared with any of the latencies in study. Although the interelectrodal distances have a great variance, the difference in the magnitude of the latencies is consistent. Values expressed in msec and obtained from preparations stimulated at low frequencies (1.0/sec).*

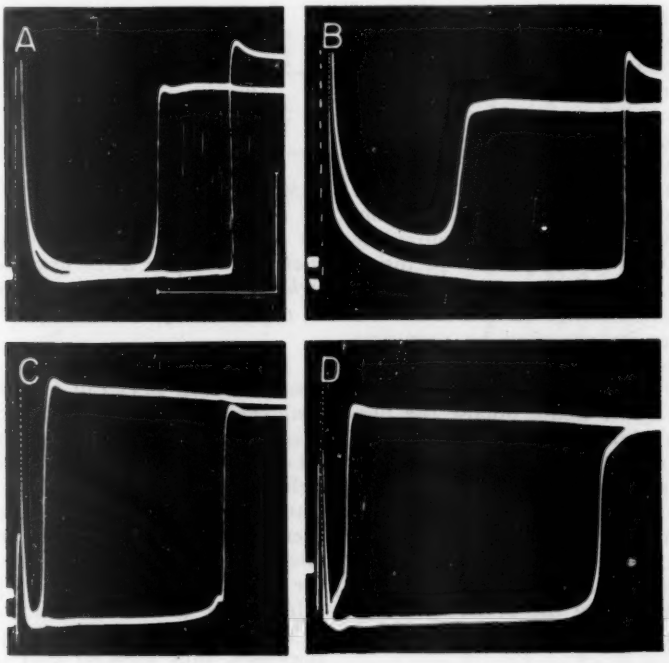

FIG. 7. — *Variations of the M-P and P-M latencies when NaCl of the Tyrode solutions was diminished (50 %). A and C as in fig. 6. B and D, 12 minutes after perfusion with Tyrode of low NaCl concentration. When normal Tyrode is replaced, the magnitude of the latencies came down to the control values. Calibrations: 30 msec and 50 mV.*

show further that these junctions are polarized. That the magnitude of the latency depends on the direction in which the impulses travel M-P or P-M, is shown by the following data. a) As the Table I shows, the values of the P-M latency were always greater than those of M-P latency. This fact was rather unexpected; since normally the activation of the ventricular muscle is achieved through the Purkinje network, one would expect the propagation from Purkinje fibers to myocardium to be faster than the propagation in the opposite direction. b) Under certain experimental conditions, impulses propagated only in one direction. Each tissue stimulated directly elicited its corresponding response, showing that the unidirectional block was not due to a failure to conduct impulses in one of the tissues, but probably to changes occurring at the junction. c) When a Purkinje fiber was impaled by a stimulating micro-electrode, and another recording micro-pipette was introduced into a myocardial cell; it was possible to register a long latency of the corresponding M response. Interchanging the stimulating and recording electrodes, in such a manner that the myocardial cell was electrically stimulated, and therefore the impulse travelled in the opposite direction, the latency of initiation

for the Purkinje potential was briefer than in the former case (fig. 4). d) Short periods of asphyxia lengthened the P-M latency earlier and to a larger extent than the M-P latency. This lengthening could lead to the block of the P-M propagation, which was in some cases irreversible. e) The same phenomenon could be observed when the NaCl concentration was diminished or quinidine sulphate was added to the perfusion chamber.

The lengthenings of both M-P and P-M latencies provoked by quinidine may partly explain the mechanism of action of this drug as an antifibrillatory agent, since it would oppose the propagation of impulses from Purkinje to muscle and vice versa. This explanation is in agreement with Hoffman's (1958) who interpreted the action of quinidine as due to a lengthening of the refractory period of both types of fibers. Our results did not afford any definite conclusion regarding the preferential site in which the drug acts.

Evidence in favour of a functional discontinuity between Purkinje fibers and myocardial cells

The present observations show the existence of a delay of considerable duration between the activation of the specialized conducting tissue and the myocardium. The characteristics of the propagation of impulses through this system suggest the possibility of a functional discontinuity between the two cardiac fibers. The following facts support this suggestion. 1) There is a long latency in the responses of the Purkinje and myocardial cells, when one of the tissues is activated by the other. 2) This latency is longer for P-M than for M-P propagation. 3) The latencies cannot be explained by long pathways or low conduction velocity, since they appear even between two adjacent cells, 15-30 μ apart. 4) Each fiber can follow higher frequencies when stimulated directly than when activated through the other set of fibers (see Table II). 5) Asphyxia, reduction in the external NaCl concentration and quinidine, all produce lengthenings of both the M-P and P-M latencies, their action being greater on the latter. When these factors produce a block, the direct stimulation of each of the tissues evokes the corresponding responses, thus showing that the propagation within the Purkinje and myocardial fibers is not impaired.

TABLE II

STIMULATION

DIRECT		INDIRECT	
P-7.3	M-8.1	P-5.7	M-4.7

Maximal frequencies (F/sec) followed by the papillary muscle cells (M) and by the Purkinje fibers (P) when stimulated directly or when activated through the other tissue (average values of 14 experiments).

A discontinuity between Purkinje cells and ventricular muscle fibers has been postulated on the basis of several histological studies (Glomset and Glomset, 1940; Wakefield and Chandler, 1924; Abramson and Margolin, 1936; González-Ramírez, 1960). Since other histological reports, however, support the opposite view (Kugler and Parkin, 1956; Nonidez, 1943; Truex and Copenhaver, 1947) it is not possible at present to draw any definite conclusions based on histology.

Source of the latencies

Since the mechanism of the propagation of impulses in cardiac tissues, is probably electrical, a first approach to explain the M-P and P-M latencies

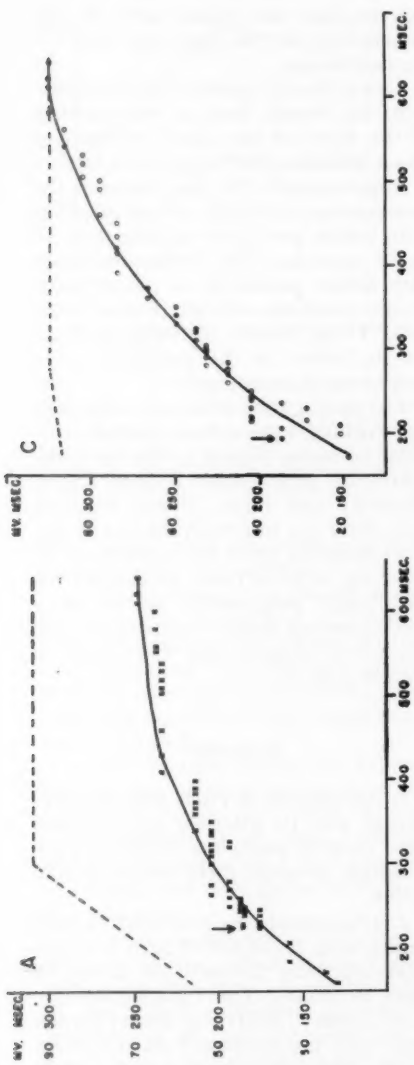

FIG. 8. — *A. Changes of the duration (crosses) and of the amplitude (broken line) of the papillary muscle cell transmembrane potential when it was stimulated with progressively increasing frequencies. C. Same as A but for the Purkinje fibers electrogram. Ordinates, duration of potentials (msec, right scale) and amplitude (mVolts, left scale). Abscissae, intervals between stimuli in msec (reciprocal of the frequency). The arrows show the maximal frequency followed by the indirectly activated tissue. Although these changes occurred simultaneously with the lengthenings of the M-P and P-M latencies (curves B and D respectively) they do not seem to be determinant of these latencies under basal conditions (low frequencies) in which both duration and amplitude have a proper magnitude. In this case the impulses followed higher frequencies when travelling in the P-M direction (5.2/sec) than in the opposite direction M-P (4.3/sec). See curves B and D.*

would be to analyse the properties of the potentials generated by these structures. The important diminution of the amplitude and duration of the action potentials produced by high frequency stimulation is concomitant with the lengthening of the M-P or P-M latencies (figs. 5 and 8). Similar results were obtained when the external NaCl was reduced, when quinidine was added or asphyxia was provoked (figs. 6, 7 and 9). The influence of these factors was

studied because it is well known that they induce changes of amplitude and duration of the electrogram of several tissues (Draper and Weidman, 1951; Hoffman and Suckling, 1954; Hodgkin and Katz, 1949; Johnson, 1956; Alanís, López and Pulido, 1959a). According to the electrical theory of propagation both amplitude and duration are of importance for the activation of excitable structures. Although in all cases a lengthening of the M-P or P-M delay was observed when the amplitude, duration and rate of depolarization of both potentials decreased, these changes do not explain the long latencies observed with slow frequencies of stimulation (0.5/sec), at which neither the amplitude nor the duration of the action potentials are modified.

A possible mechanism for the long delays, could be a slow conduction velocity of the impulse within the junctional area. However, the conduction velocity would have to be extremely slow in order to explain the latencies which appear even with short conduction distance (10-30 μ). It also seems improbable that the conduction velocity of the Purkinje fibers (4-5 m/sec) would suddenly fall importantly (about 0.001 m/sec) in the junctional zone and increase again when the impulses reach the myocardium (0.4-0.5 m/sec). It appears therefore that the delays which occur in the Purkinje-myocardium junctions are due to a stop of the impulses in this regions and not to a slow conduction. As regards the mechanism for this stopping, one can only tentatively suggest that it may be similar to those which occur in the various types of synapses or ephapses.

The failure of the impulses to propagate to the distal tissue, when high frequencies of stimulation are applied, is not due to the refractoriness of either set of fibers, since each one follows higher frequencies when stimulated directly (see Table II). This fact again suggests that the critical zone for the propagation is the junction between the two tissues.

Alvarez-Buylla (personal communication) has found, that in the ventricle of the heart of the chick embryon of about 72 hours, there appears a latency of approximately 30 msec between the spontaneous activation of two adjacent cells, which are clearly separated by 10 to 15 microns. This latency occurred with action potentials of proper magnitude, duration and rate of depolarization. These results, although indirect are in favour of the possibility of a membrane discontinuity.

The propagation of impulses through the Purkinje-myocardium system is similar in many aspects to the auriculoventricular propagation (Alanís, López, Mandoki and Pilar, 1959b). In both cases there are important delays within short distances when the impulses travel from one type of tissue to another. In both cases propagation occurs in a heterogeneous system (contractile and conductive tissues) and can occur in both directions.

Summary

1. An isolated and perfused papillary muscle and its attached false tendon were used to study the temporal relationships between their electrical activation.

2. Transmembrane potentials of both tissues were registered. There is a considerable delay between the action of these structures. The Purkinje-myocardium latency (P-M) was always greater than the myocardium-Purkinje delay (M-P). Their magnitude was 29 and 18 msec respectively, and they occurred even between action potentials of adjacent cells (15-30 μ).

3. The long delay was not due to slow conduction velocity in any of the two tissues, since their cumulative conduc-

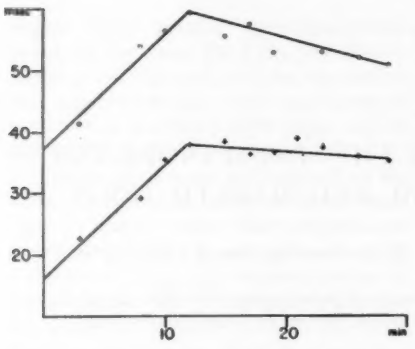

FIG. 9. — *Action of quinidine sulphate (200 μg) on the M-P (crosses) and on the P-M (circles) latencies. Abscissa, time in minutes after quinidine was added to the chamber. Ordinates, magnitude of the latencies in msec.*

tion times was considerably smaller than the observed latencies.

4. These latencies appeared at any frequency of stimulation (0.5-4.7 per sec). Progressively increasing frequencies lengthened both the M-P and the P-M latencies. Concurrently, the amplitude and duration of the action potentials decreased.

5. The limiting factors for propagation do not seem to be in the tissues themselves, since each type of fiber, when stimulated directly, followed higher frequencies (7.7 per sec) than when activated through the other tissue (5.2 per sec).

6. Other experimental variables, of different nature, such as asphyxia, diminution of the external concentration of NaCl and quinidine provoked a lengthening of both latencies and in some cases unidirectional block of the impulses.

7. These data suggest the existence of a functional discontinuity between the Purkinje and ventricular muscle cells. The propagation of impulses through these junctions is similar to that through the A. V. node.

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EFFECT OF GLUCAGON ON THE SERUM INORGANIC PHOSPHORUS OF NORMAL AND DIABETIC DOGS

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Kirtley, Waife and Peck (1) were the first to report on the effect of glucagon on the serum inorganic phosphorus. In four "stable" diabetics, the blood sugar arose approximately at the same level as in normal subjects, remaining high for a greater length of time; it was found in this group a defect in the lowering of the serum inorganic phosphorus. In four "unstable" diabetics, sensitive to insulin, a lesser increase in blood sugar was obtained than in normal cases and the original figures were returned to in approximately the same length of time as the latter; phosphorus decreased in a normal way in this second group. In another report, Kirtley & col. (2) complement the aforementioned data and also indicate analagous differences to those found in glucagon using epinephrine.

In a previous report (3) we have demonstrated that in cases of diabetes which do not respond with a decrease in the serum inorganic phosphorus to glucose administered intravenously, this decrease is plainly evident afer injecting glucagon in the same manner. In the cases of diabetics who respond to glucose the effect of glucagon is also well defined. It was thus concluded that glucagon must have its own effect on the serum inorganic phosphorus independentlv of that occasioned by the liberated insulin as an answer to hyperglycemia.

In the investigations which we have reported here the action of glucagon on the blood sugar and the serum inorganic phosphorus were studied in pancreatectomized and alloxanized dogs to experimentally discard the participation of insulin in the decrease in serum phosphate found after the injection of the pancreatic hyperglycemic factor.

Material and Methods. – Apparently normal dogs were used weighing from 10 to 21 kg. Alloxan diabetes was induced by administering alloxan in doses of 60 to 80 mg per kg of weight by vein. In some dogs it was necessary to repeat alloxan injection for obtaining the desired effect.

The pancreatectomized dogs were operated on employing the technique used by Dr. Charles H. Best which was graciously supplied by Dr. Ana Sirek. The pancreatectomized dogs were fed with a daily supply of raw pancreas as well as their usual meals.

The diabetic dogs were maintained

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under Lente Insulin (generously supplied by the firms Eli Lilly and Novo). Insulin was discontinued the day before the experiment and only those animals which had fasting blood sugar values above 225 mg/100 ml were used.

The animals were accustomed to the vein punctures in order to avoid reactions of fear or anger. The weights were watched to assure a normal and stable weight and frequent determinations of blood sugar were performed to observe the degree of diabetic control. The dogs were under fasting from the previous day of the experiment (approximately 20 hours). The experiments of spontaneous variation were done collecting blood every 15 minutes during 60 minutes. Glucose tolerance tests were performed by injecting into the vein 1 ml of 3 M dextrose solution per kg of weight at an approximate rate of 10 ml/minute. Prior to this, a blood sample was taken and afterwards samples were taken every 15 minutes after the injection, for one hour. The technique employed for the tests with glucagon was the same, but instead of dextrose, a solution of crystalline glucagon was used at a dosis of 0.01 ml per kg containing 1 mg/ml. Glucagon was generously supplied by Eli Lilly Co. The determinations of glucose were done in duplicate with the Nelson [4] procedure and those of serum inorganic phosphorus, also in duplicate, with the Fiske and Subbarow [5] method. Readings were carried out with a Coleman Jr. spectrophotometer for the phosphorus and a Klett photocolorimeter for the blood sugar. The statistical significance was established by the "t" test.

Results

The results obtained are summarized in Tables 1 to 6.

Discussion

From an analysis of the results the following conclusions can be deduced. In normal dogs the glucose as well as the glucagon determine an evident decrease of the serum inorganic phosphorus statistically significant when compared with the spontaneous variation. In pancreatectomized and alloxanized dogs the glucose administration determines insignificant decreases; whereas the glucagon produces marked and significant changes. It is convenient to stress, nevertheless, that the decrease of the phosphorus with glucagon is more pronounced in normal dogs than in those with diabetes. The differences in the response to glucose as well as glucagon between the two types of diabetic dogs was not significant.

From the aforementioned data it may be concluded that glucagon has its own effect on the serum inorganic phosphorus independently of the reactional secretion of insulin related to the elevation of blood sugar. The mechanism of this effect has not as yet been determined. It is possible that it depends, at least in part, on the utilization of phosphorus by the liver for the formation of glucose-1-phosphate from the glycogen. Nevertheless, Elrick & col. [6, 7] have observed an increase in renal excretion of inorganic phosphorus after the administration of glucagon.

With reference to the blood sugar values, the initial increase found in 15 minutes under the influence of glucagon was greater in normal dogs than in the pancreatectomized and alloxanized ones; in 60 minutes, as was to be expected, in normal dogs there is a return to almost the initial values, while in the case of diabetics of both groups the blood sugar is still high, the difference being significant with respect to the normal ones only in the pancreatectomized dogs.

TABLE I

Means and standard errors of the changes of serum inorganic phosphorus produced by Dextrose and Glugacon in Normal, Pancreatectomized and Alloxanized Dogs

GROUPS	Nº of Exp.	Initial Values	Minutes 15	30	45	60
Normal Dogs						
Spontaneous variations	14	3.59 ± .20	+ .05 ± .05	— .02 ± .05	+ .02 ± .06	.00 + .05
Dextrose	12	4.90 ± .25	— .36 ± .07	— .60 ± .09	— .64 ± .11	— .57 ± .12
Glucagon	12	3.68 ± .14	— .64 ± 0.6	— .80 ± .09	— .54 ± .07	— .41 ± .10
Pancreatectom. dogs						
Spontaneous variations	26	4.19 ± .21	— .01 ± .04	— .16 ± 0.7	— .30 ± 0.8	— .28 + .10
Dextrose	18	4.26 ± .19	— .09 ± .03	— .11 ± .05	— .14 ± .06	— .15 ± .08
Glucagon	20	4.75 ± .25	— .45 ± .05	— .64 ± .07	— .51 ± .09	— .43 ± .09
Alloxanized dogs						
Spontaneous variations	24	3.90 ± .18	— .08 ± .04	— .19 ± .05	— .25 ± 0.7	— .27 ± .05
Dextrose	20	3.94 ± .24	— .08 ± .04	— .17 ± .05	— .28 ± .08	— .32 ± .09
Glucagon	24	3.99 ± .19	— .46 ± .06	— .57 ± .06	— .44 ± .08	— .32 ± .07

TABLE II

Statistical significance of the differences of the changes of serum inorganic phosphorus within each group

P VALUES

	Initial values	Minutes 15	30	45	60
Normal dogs					
Spontaneous variation vs. dextrose	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Spontaneous variation vs. glucagon	0.7	< 0.01	< 0.01	< 0.01	< 0.01
Pancreatectomized dogs					
Spontaneous variation vs. dextrose	0.8	0.1	0.6	0.1	0.3
Spontaneous variation vs. glucagon	0.1	< 0.01	< 0.01	0.1	0.3
Alloxanized dogs					
Spontaneous variation vs. dextrose	0.9	< 0.9	0.8	0.8	0.6
Spontaneous variation vs. glucagon	0.7	< 0.01	< 0.01	0.05	0.6

TABLE III

Statistical significance of the differences of the changes of serum inorganic phosphorus between groups

P VALUES

		Initial values	Minutes 15	30	45	60
Spontaneous variation						
Normal dogs	vs. pancreatectom.	0.05	0.3	0.1	< 0.01	0.01
Normal dogs	vs. alloxanized dogs	0.3	0.02	0.02	< 0.01	< 0.01
Pancreatectomized dogs	vs. alloxanized dogs	0.3	0.2	0.7	0.7	< 0.9
Dextrose						
Normal dogs	vs. pancreatectom.	0.05	< 0.01	< 0.01	< 0.01	< 0.01
Normal dogs	vs. alloxanized dogs	< 0.01	< 0.01	< 0.01	< 0.01	0.1
Pancreatectomized dogs	vs. alloxanized dogs	0.3	0.8	0.4	0.2	0.2
Glucagon						
Normal dogs	vs. pancreatectom.	< 0.01	0.02	0.2	0.8	0.9
Normal dogs	vs. alloxanized dogs	0.2	0.02	0.02	0.3	0.5
Pancreatectomized dogs	vs. alloxanized dogs	0.01	0.9	0.4	0.6	0.3

TABLE IV

Means and standard errors of the changes of blood sugar produced by dextrose and glucagon in normal, pancreatectomized and alloxanized dogs

GROUPS	Nº of Exp.	Initial values	Minutes 15	30	45	60
Normal dogs						
Spontaneous variations	14	66 ± 2	+ 1 ± 1	+ 1 ± 1	+ 2 ± 1	+ 3 ± 2
Dextrose	14	74 ± 2	+ 76 ± 8	+ 20 ± 9	+ 3 ± 4	— 2 ± 2
Glucagon	12	71 ± 3	+ 59 ± 4	+ 33 ± 6	+ 13 ± 4	+ 8 ± 3
Pancreatectomized dogs						
Spontaneous variations	28	335 ± 14	+ 5 ± 3	+ 8 ± 4	+ 7 ± 4	+ 0 ± 4
Dextrose	18	321 ± 18	+ 103 + 6	+ 87 ± 4	+ 71 ± 6	+ 59 ± 6
Glucagon	25	339 ± 16	+ 47 ± 6	+ 61 ± 7	+ 50 ± 8	+ 33 ± 8
Alloxanized dogs						
Spontaneous variations	30	263 ± 7	+ 6 ± 2	+ 7 ± 3	+ 10 ± 4	+ 9 ± 3
Dextrose	20	305 ± 12	+ 114 ± 7	+ 85 ± 6	+ 66 ± 6	+ 51 ± 7
Glucagon	24	280 ± 8	+ 30 ± 4	+ 38 ± 4	+ 31 ± 6	+ 17 ± 5

TABLE V

Statistical significance of the differences of the changes of blood sugar within each group

P VALUES

	Initial values	Minutes 15	30	45	60
Normal dogs					
Spontaneous variation vs. dextrose	< 0.01	< 0.01	0.05	0.8	0.1
Spontaneous variation vs. glucagon	0.2	< 0.01	< 0.01	0.01	0.2
Pancreatectomized dogs					
Spontaneous variation vs. dextrose	0.5	< 0.01	< 0.01	< 0.01	< 0.01
Spontaneous variation vs. glucagon	0.8	< 0.01	< 0.01	< 0.01	< 0.01
Alloxanized dogs					
Spontaneous variation vs. dextrose	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Spontaneous variation vs. glucagon	0.1	< 0.01	< 0.01	< 0.01	0.2

TABLE VI

Statistical significance of the differences of the changes of blood sugar between groups

P VALUES

	Initial values	Minutes 15	30	45	60
Spontaneous variation					
Normal dogs vs. pancreatect. dogs	< 0.01	0.2	0.1	0.2	0.5
Normal dogs vs. alloxanized dogs	< 0.01	0.02	0.05	0.05	0.1
Pancreatectomized dogs vs. alloxanized dogs	< 0.01	0.8	0.8	0.6	0.1
Dextrose					
Normal dogs vs. pancreatect. dogs	< 0.01	0.01	< 0.01	< 0.01	< 0.01
Normal dogs vs. alloxanized dogs	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pancreatectomized dogs vs. alloxanized dogs	0.5	0.2	0.8	0.6	0.1
Glucagon					
Normal dogs vs. pancreatect. dogs	< 0.01	0.1	< 0.01	< 0.01	< 0.01
Normal dogs vs. alloxanized dogs	< 0.01	< 0.01	0.5	0.01	0.1
Pancreatectomized dogs vs. alloxanized dogs	< 0.01	0.02	< 0.01	0.05	0.1

Summary

We have studied in this report the effect of glucagon on the serum inorganic phosphorus and the blood sugar, comparing the results with those of the intravenous administration of glucose in normal, pancreatectomized and alloxanized dogs. It was found that glucagon decreases the serum inorganic phosphorus in the three types of animals and that glucose only affects the normal ones. It is concluded that glucagon has its own effect on the serum inorganic phosphorus independent of the reactional secretion of insulin that hyperglycemia produces under normal conditions. The effect of glucagon on the blood sugar is greater in 15 minutes in normal dogs, but in diabetics the hyperglycemia tends to be more prolonged.

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SEXUAL DIFFERENCE IN THE I ^{131} UPTAKE BY THE SUBMAXILLARY GLANDS IN A_2G MICE

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It is known that besides the thyroid there are several organs that have the ability to concentrate iodine in greater amounts than plasma, namely the gastric mucosa ([4, 6, 19]), the small intestine ([18]), the mammary glands ([6]), the placenta ([15]) and the salivary glands ([2, 6, 15, 19, 21]).

The ability of the salivary glands to concentrate iodine varies with each gland and with the species. Thus, none of these glands can do it in the rat, whereas in mice and hamsters the submaxillary glands concentrate iodine while the sublingual glands cannot concentrate it ([17]).

The submaxillary glands of several inbred strains of mice present a marked sexual dimorphism ([10]), the epithelium of the alveolar ducts is high and cylindric in the males and low and flat in the females and in the castrated animals ([10, 11, 12]). This fact induced us to study the uptake of I^{131} by the salivary glands in A_2G mice and we found that the uptake by the submaxillary glands was much higher in males than in females ([7]). This observation prompted these further studies reported here.

Other workers ([16]) have also encountered a sexual difference in the I^{131} concentration by the submaxillary glands in C_3H mice.

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Material and Methods

The animals used were A_2G mice, of 20 to 30 grams weight, from the colony of the "Centro de Endocrinología" directed by Dr. Eva I. Saurer. These animals descend from those sent to us by Dr. C. Lane-Potter of the Laboratory Animals Centre, Carshalton, Surrey, England.

The I^{131} employed came from the Radiochemical Centre, Amersham, England and it was supplied to us by the "Comisión Nacional de la Energía Atómica" of the Argentine Republic.

In all the experiments each animal received an intraperitoneal injection of 6 microcuries of I^{131}; the mice were killed at different intervals of time, according to the experiment. At autopsy the thyroid and the salivary glands were disected and weighed. The blood, obtained by heart incision, was centrifuged and 0.1 ml of serum was used to measure its radioactivity in a well counter. The thyroid was placed in 10 ml of a 20 % solution of NaOH and the activity of 1 ml measured. As to the salivary glands, their radioactivity was evaluated by a well counter; the organs

were pressed to the bottom of tubes specially designed for the well counter. All the samples were measured in a well counter connected to a scaler, both of them made by Nuclear Chicago.

The counts were used to calculate the I^{131} uptake by the thyroid and the $\frac{\text{mg of salivary gland}}{\mu\text{l of serum}}$ ratio of all the salivary glands: submaxillary (SM/S), sublingual (SL/S) and parotid (P/S).

In some of the experiments castrated mice were used, 1 to 3 months after castration. Some animals, males or females, castrated 1 to 2 months before, received during 1 month a daily subcutaneous injection of 0.1 ml of a solution in sterile peanut oil of testosterone propionate or of estradiol benzoate or dipropionate.

At the end of the experiments it was calculated the mean and the standard error; and for comparison of the means we used the "t" index to determine the probability in the table of Fisher and Yates.

Results

Sexual difference

Five experiments were performed in which the I^{131} uptake by the salivary glands was compared in groups of male and female mice of the same age, except in the first one where the females were younger.

The mice were killed at different time intervals after getting the tracer namely at 1, 2, 4, 8 or 24 hours. The results are reported in Table I.

In all the experiments the thyroids were of similar size in males and in females, but the I^{131} uptake was higher in the latter, the difference not being significant in experiments 1, 2 and 4 and slightly significant ($p < 0.05$) in experiments 3 and 5. Apparently there is a small or no sexual difference in the thyroid function.

The parotid glands were smaller in the females than in the males, but the amount of I^{131} per mg (P/S ratio) was the same in both sexes. There were no major differences in the size of the sublingual glands, nor in the ability to take-up I^{131} (SL/S ratio) except in experiment 2 that was slightly greater in the males ($p < 0.05$). We can see that the parotids do not concentrate I^{131} and that the sublinguals concentrate in a ratio 2/1 or 3/1, but that there is no consistent sexual difference as to the I^{131} in either one.

The main difference is in the submaxillary glands. These were always bigger in the males than in the females, which is in accordance with the sexual difference in height of the epithelium of the alveolar ducts, namely cylindric in the males and flat in the females. The uptake of I^{131} per mg of gland (SM/S ratio) was significantly higher in the males ($p < 0.001$) killed at 4, 8 and 24 hours, and higher but less significant in experiments 1 ($p < 0.10$) and 2 ($p < 0.05$).

It is evident that the submaxillary glands of A_2G mice concentrate markedly I^{131} in proportions that vary according to the experiment. There is a sexual difference favoring the males, which is small at 1 hour but very great at 4, 8 and 24 hours. In experiment 3 the concentration of I^{131} by the submaxillary glands of the males in relation with serum was 25 to 1.

Effects of castration

Two experiments were carried out: 33 days elapsed in experiment 6 after castration of males and females, and 36 days was the interval in experiment 7. In both of them the animals were killed 4 hours after the tracer dose. The results are shown in Table II.

We found neither alteration in the I^{131} uptake by the thyroid due to cas-

TABLE I •

Sexual difference in the I^{131} uptake by the salivary glands in A_2G mice

Experiment Nº	Time after I^{131} tracer hours	Animals			Thyroid		Submaxillary		Sublingual		Parotid	
		Sex	No	Weight (g)	Weight (mg)	I^{131} uptake % (M ± SE)	Wt. (mg)	$SM_{/S}$ (M ± SE)	Wt. (mg)	$SL_{/S}$ (M ± SE)	Wt. (mg)	$P_{/S}$ (M ± SE)
1	1	M	12	27	2.1	13.1 ± 1.8	112	6.2 ± 0.5	20	3.4 ± 0.9	130	0.7 ± 0.2
		F	11	17	2.2	8.9 ± 1.3	49	4.6 ± 0.8	14	3.4 ± 0.5	68	0.8 ± 0.06
2	1	M	9	20	2.7	28 ± 3.9	126	2.0 ± 0.3	19	2.0 ± 0.2	119	0.5 ± 0.2
		F	10	19	2.8	39 ± 5.0	78	1.3 ± 0.1	19	1.2 ± 0.1	81	0.4 ± 0.2
3	4	M	10	21	3.1	13 ± 1.5	75	24.9 ± 4	12	1.7 ± 0.4	68	0.7 ± 0.06
		F	10	19	3.3	20 ± 2.3	39	6.4 ± 1.5	13	2.3 ± 0.3	56	0.5 ± 0.03
4	8	M	8	20	1.9	24 ± 3.1	93	12.8 ± 1.7	17	1.7 ± 0.2	102	1.6 ± 0.5
		F	11	17	2.0	28 ± 3.5	60	5.7 ± 0.5	11	2.6 ± 0.6	69	1.4 ± 0.2
5	24	M	9	24	2.2	12 ± 3.2	105	8.6 ± 2.4	17	1.7 ± 0.4	108	0.8 ± 0.2
		F	10	21	2.7	21 ± 3.3	73	1.0 ± 0.1	12	0.9 ± 0.3	72	0.9 ± 0.4

TABLE II

Effects of castration upon the I^{131} uptake at 4 hours by the salivary glands of A_2G Mice

Experiment Nº	Animals			Castration	Thyroid		Submaxillary		Sublingual		Parotid	
	Nº	Sex	Wt.		Wt. (mg)	I^{131} uptake % (M ± SE)	Wt. (mg)	$SM_{/S}$ (M ± SE)	Wt. (mg)	$SL_{/S}$ (M ± SE)	Wt. (mg)	$P_{/S}$ (M ± SE)
6	7	M	24	no	—	—	90	18.0 ± 1.8	19	3.2 ± 0.8	71	0.9 ± 0.2
	6	M	26	yes	—	—	77	17.9 ± 4	20	3.4 ± 0.9	90	0.7 ± 0.1
	6	F	23	no	—	—	44	6.1 ± 1.1	11	2.8 ± 0.8	54	0.6 ± 0.08
	5	F	23	yes	—	—	33	8.5 ± 2.0	11	3.2 ± 1.0	57	0.8 ± 0.1
7	7	M	25	no	3.1	7 ± 1.4	141	5.9 ± 1.1	21	11 ± 1.5	145	1.4 ± 0.3
	10	M	24	yes	2.9	5 ± 0.9	115	3.2 ± 0.5	23	3.1 ± 0.6	132	1.0 ± 0.1
	10	F	22	no	2.6	5 ± 1.2	107	4.3 ± 0.7	21	6.1 ± 1.1	117	1.4 ± 0.2
	7	F	23	yes	3.7	8 ± 1.5	95	3.0 ± 0.6	20	2.9 ± 0.3	118	1.1 ± 0.2

tration nor sexual difference. Likewise, neither alteration nor difference was seen in the parotids in both experiments.

As to the sublingual glands, there was no sexual difference in the concentration of I^{131} nor was it altered by castration in experiment 6; but in experiment 7 there was a significant sexual difference favoring the males ($p < 0.01$) and castration diminished it significantly in the males ($p < 0.001$) and slightly in the females ($p < 0.02$). There was no difference in the size of these glands in normal and castrated ones.

Regarding the submaxillary glands, experiment 6 showed a pronounced sexual difference ($p < 0.001$), but castration did not alter the I^{131} concentration either in males or in females.

In experiment 7, whereas no significant sexual difference was found ($p < 0.5$), castration decreased the uptake slightly significant in males ($p < 0.05$) and not significantly in females ($p < 0.2$).

Action of testoterone propionate

In experiments 8 and 9, the effect of castration and the action of testosterone propionate in castrated mice are simultaneously compared. In experiment 8 —male and female mice— and in experiment 9 —male animals— a comparative study is carried out of intact animals, castrated animals and castrated animals injected daily with 10 to 100 micrograms of testosterone propionate.

In experiment 8, 52 days elapsed since castration and the injections were given during 39 days, whereas in experiment 9, 31 days passed before starting the course of injections of 37 days.

The animals were killed on the day of the last injection, 4 hours after receiving the tracer dose of I^{131}.

The results are shown in Table III.

Neither castration nor the administration of testosterone propionate have shown any influence on the uptake of I^{131} by the thyroid. Castration did not affect the I^{131} concentration by the parotid and sublingual glands. Testosterone propionate did not act upon the concentration of I^{131} by these organs in experiment 8 but did increase it in experiment 9 by the parotids ($p < 0.01$) but not by the sublinguals ($p < 0.5$).

Experiment 8 confirms the sexual difference in the concentration of I^{131} by the submaxillary glands ($p < 0.001$). In both experiments castration decreased the I^{131} concentration by them in males, very significantly in experiment 8 ($p < 0.01$) and slightly less so in experiment 9 ($p < 0.05$), but the females showed no difference. The period after castration was respectively 91 and 68 days, and the decrease in the size of the glands was 53% and 27%.

Testosterone propionate caused a markedly significant rise in I^{131} concentration by the submaxillary glands in castrated male mice —in experiment 8 ($p < 0.001$) and in experiment 9 ($p < 0.02$)— and the increase was higher than in non castrated male controls in the same experiment. The increase produced by this substance in castrated females was not significant ($p < 0.5$).

These experiments confirm that castration lowers the concentration of I^{131} by the submaxillary glands in males and that testosterone propionate raises it in a degree that may exceed that of intact male controls.

Action of estradiol benzoate or dipropionate

Experiments 10 and 11 were designed to study the effects of estrogens upon the concentration of I^{131} by the salivary glands of castrated mice.

In experiment 10 estradiol benzoate

TABLE III

Action of testosterone propionate upon the I^{131} uptake at 4 hours by the salivary glands of castrated A_2G Mice

Experiment Nº		Animals			Castration	Thyroid		Submaxillary		Sublingual		Parotid	
	Dose μg	Nº	Sex	Wt (g)		Wt. (mg)	I^{131} uptake % (M ± SE)	Wt. (mg)	$SM_{/S}$ (M ± SE)	Wt. (mg)	$SL_{/S}$ (M ± SE)	Wt. (mg)	$P_{/S}$ (M ± SE)
8	0	6	M	27	no	1.2	10 ± 1.8	115	31.1 ± 2.1	15	3.5 ± 0.2	99	1.2 ± 0.2
	0	6	M	25	yes	0.8	8 ± 0.9	54	17.1 ± 2.7	12	3.7 ± 1.3	84	1.5 ± 0.2
	100	5	M	26	yes	1.1	11 ± 1.4	113	46.4 ± 1.2	13	4.0 ± 0.9	103	1.9 ± 0.5
	0	5	F	18	no	1.1	9 ± 3.0	38	10.0 ± 1.8	11	4.2 ± 0.6	67	1.3 ± 0.3
	0	5	F	22	yes	1.3	10 ± 2.0	54	8.5 ± 1.2	15	4.1 ± 0.5	79	1.1 ± 0.1
	10	8	F	25	yes	1.3	8 ± 1.0	73	7.5 ± 1.4	17	4.0 ± 0.4	88	1.1 ± 0.1
	100	4	F	23	yes	1.2	8 ± 1.1	98	10.0 ± 1.2	17	3.6 ± 0.5	91	2.0 ± 1.0
9	0	10	M	25	no	2.9	4 ± 0.9	118	14.7 ± 1.7	15	3.2 ± 1.2	121	0.7 ± 0.06
	0	10	M	24	yes	0.8	3 ± 0.7	86	10.4 ± 1.2	14	4.2 ± 0.5	111	0.6 ± 0.05
	100	10	M	21	yes	0.8	3 ± 0.8	73	18.6 ± 2.6	11	7.3 ± 2.1	90	1.3 ± 0.2

was used in castrated males and females, and in experiment 11 estradiol dipropionate in castrated males.

In experiment 10 the injections started 7 days after castration and continued for 39 days, whereas in experiment 11 they began 39 days after castration and went on for 38 days.

The animals were killed on the day of the last injection, 4 hours after receiving a tracer dose of I^{131}. The results are reported in Table IV.

Neither castration nor the administration of estrogens bore any significant influence upon the I^{131} uptake by the parotids or sublinguals.

Experiment 10 is a confirmation of the sexual difference in the I^{131} concentration by the submaxillary glands ($p < 0.01$). Castration caused a small decrease in the males in experiment 10 and none in the females in experiment 10 nor in the males in experiment 11. Estradiol benzoate or dipropionate had no effect on the I^{131} concentration by the submaxillary glands of castrated male or female mice.

Influence of the time since castration

In Table V are summarized the six experiments in which the concentration of I^{131} by the submaxillary glands in intact and castrated male mice was studied. It is known that the atrophy of the epithelium of the alveolar ducts caused by castration is developed slowly and is enhanced by the time elapsed since.

In this way we may compare the decrease in I^{131} produced by castration with the time pased since this operation and with the degree of atrophy of the submaxillary glands measured by the percentage of decrease in the weight of the glands of castrated mice compared with those of normal animals.

In experiments 6 and 11 there was no difference in concentration of I^{131} by the submaxillary glands in normal and castrated mice. The times since castration were 33 and 68 days, and the percentage of submaxillary atrophy 14% and 10%; but the difference in weight of the submaxillary glands in normal and castrated animals was not significant.

In experiment 10 the difference in I^{131} concentration in normal and castrated mice was not significant. The time since castration was 46 days and the percentage of submaxillary atrophy 5%, but there was no significant difference in weight of the submaxillary glands in intact and castrated animals.

In experiments 7 and 9 the difference in concentration of I^{131} between normal and castrated animals was significant ($p < 0.05$). The time since castration was respectively 36 and 68 days and the percentage of submaxillary atrophy 19% and 27%; the difference in weight in normal and castrated mice was significant ($p < 0.01$; < 0.02).

In experiment 8 the difference in concentration of I^{131} between the submaxillary glands in normal and castrated animals was significant ($p < 0.01$). The time since castration was 91 days and the submaxillary atrophy 53% with a significant difference ($p < 0.001$) in the weight of this gland in intact and castrated mice.

These experiments would demonstrate that castration reduces the I^{131} uptake by the submaxillary glands in male mice if sufficient time is allowed since operation to bring about a significant difference in weight between the glands of intact and castrated animals, equivalent to a decrease of 20% or more in the weight of these glands.

Discussion

It is a matter of discussion the role of the salivary glands in the metabolism of iodine ([17]). It is known that they

TABLE IV

Action of estrogens upon the I^{131} concentration at the 4 hours by the salivary glands of A_2G castrated mice

Experiment Nº	Treatment	Dose	Castration	Animals			Thyroid		Submaxillary		Sublingual		Parotid	
		(μg)		Nº	Sex	Wt. (g)	Wt. (mg)	I^{131} uptake % (M ± SE)	Wt. (mg)	$SM_{/S}$ (M ± SE)	Wt. (mg)	$SL_{/S}$ (M ± SE)	Wt. (mg)	$P_{/S}$ (M ± SE)
10	Estradiol benzoate	0	no	5	M	29	3.7	17 ± 5.0	95	11.1 ± 3.9	20	2.6 ± 0.5	103	1.5 ± 0.2
		0	yes	10	M	25	3.1	16 ± 5.0	90	5.7 ± 1.5	17	2.6 ± 0.5	102	1.8 ± 0.2
		10	yes	10	M	26	3.0	19 ± 3.6	90	5.6 ± 2.0	21	3.0 ± 0.4	110	1.7 ± 0.2
		0	no	5	F	21	2.3	15 ± 2.4	82	2.5 ± 0.9	17	2.1 ± 0.7	90	1.6 ± 0.4
		0	yes	10	F	24	2.9	26 ± 1.5	89	2.1 ± 1.5	24	1.8 ± 0.3	94	1.5 ± 0.2
		10	yes	7	F	22	3.5	29 ± 2.8	99	3.0 ± 0.7	20	1.9 ± 0.6	89	1.5 ± 0.2
11	Estradiol dipropio-nate	0	no	10	M	25	—	—	128	5.0 ± 0.7	19	2.3 ± 0.2	148	0.6 ± 0.08
		0	yes	8	M	25	—	—	115	4.8 ± 0.6	14	3.0 ± 0.4	124	0.7 ± 0.12
		10	yes	9	M	21	—	—	99	5.3 ± 1.2	13	2.8 ± 0.4	78	1.0 ± 0.16

have an ability to concentrate iodine in a higher degree than plasma and to secrete it into saliva, where its concentration is also higher than in plasma in dogs and in humans ([2, 8, 13, 19, 27]).

This mechanism seems to consist mainly in the active transportation of inorganic iodide and its excretion into saliva ([17]). Some workers have postulated its participation in the metabolism of organic iodine ([5, 8, 21]), notably Fawcett and Kirkwood ([15]), who refer to a special role in the degradation of diiodothyrosine and thyroxine, which has not been confirmed by others ([17, 20]).

It is also a point of interest to ascertain which cells of the salivary glands are the site of the mechanism of iodide concentration. Autoradiographic studies ([3, 14, 17]) show a greater concentration by the alveolar ducts than by the acini.

The results reported here demonstrate that the submaxillary and sublingual glands of A_2G mice concentrate I^{131} but that the parotids do not. However, the fact that draws our attention is the marked sexual difference in the I^{131} concentration by the submaxillary glands, which is higher in males than in females. This sexual difference is shared by the sublingual glands in only one experiment (Nº 7), but in the rest of them we find no such sexual difference by the sublingual or parotid glands.

Both the I^{131} concentration by the submaxillary glands and the sexual difference in this function are small at 1 hour after the tracer dose, are maximal between 4 and 8 hours and are still present at 24 hours. Not only is increased the concentration of I^{131} per mg —measured by the submaxillary/serum ratio (SM/S)— but because the submaxillary glands of males are larger the total uptake is still greater. This difference in size is associated to a histologic difference, namely the greater height of the epithelium of the alveolar ducts in male mice, composed of cylindric cells, than in females, formed by flat cells.

Castration produces in males the atrophy of the epithelium of the alveolar ducts and diminishes the I^{131} concentration by the submaxillary glands. In the females, in which castration causes either a slight atrophy or none, there is also a slight or no decrease in I^{131} concentration by the said glands.

The administration of testosterone propionate, that increases the height of the epithelium of the alveolar ducts, enhances also the I^{131} concentration by the submaxillary glands in castrated mice to a level even higher than in intact males. Estradiol benzoate or dipropionate, which fail to act upon the height of the epithelium of the alveolar ducts, do not alter the I^{131} concentration in castrated mice.

All these facts demonstrate the parallelism between the height of the alveolar ducts and the concentration of I^{131} by the submaxillary glands and they would support the hypothesis ([17]) of the role of this epithelium in the concentration of I^{131}.

We found a wide variation in the concentration of I^{131} by the salivary glands from one experiment to another, thus resulting in a SM/S ratio at 4 hours in male intact mice that ranged between 31.1 and 5. This finding prompted us to make a careful choice of the animals in order that the groups were homogeneous and that each experiment might have its own controls.

Summary

The submaxillary and sublingual glands —but not the parotids— of A^2G mice have the property of concentrating I^{131}.

There is a marked sexual difference in the concentration of I^{131} by the submaxillary glands, this ability being greater in males than in females, which

TABLE V

Relationship between the action of castration upon I^{131} uptake and upon weight of submaxillary glands in A_2G mice

Experiment Nº	Animals			Castration	Time after castration	Submaxillary						
	Nº	Sex	Wt. (g)		(days)	Weight (mg) (M ± SE)	"t"	p	Atrophy	$SM_{/S}$ (M ± SE)	"t"	p
6	7	M	24	no		90 ± 6.0	1.88	< 0.10	14 %	18 ± 1.8	0.023	< 0.5
	6	M	26	yes	33	77 ± 2.1				17.9 ± 4		
7	7	M	25	no		141 ± 7.0	2.82	< 0.02	19 %	5.9 ± 1.1	2.23	< 0.05
	10	M	24	yes	36	115 ± 6.0				3.2 ± 0.5		
8	6	M	27	no		115 ± 5.5	10	< 0.001	53 %	31.1 ± 2.1	4.09	< 0.01
	6	M	25	yes	91	54 ± 2.7				17.1 ± 2.7		
9	10	M	25	no		118 ± 6.3	2.93	< 0.01	27 %	14.7 ± 1.7	2.07	< 0.05
	10	M	24	yes	68	86 ± 8.9				10.4 ± 1.2		
10	5	M	29	no		95 ± 3.9	0.48	< 0.5	5 %	11.1 ± 3.9	1.29	< 0.3
	10	M	25	yes	46	90 ± 9.7				5.7 ± 1.5		
11	10	M	25	no		128 ± 5.7	1.58	< 0.5	10 %	5.0 ± 0.7	0.22	< 0.5
	8	M	25	yes	68	115 ± 6.8				4.8 ± 0.6		

coincides with the greater height of the alveolar ducts.

In male animals castration decreased the concentration of I^{131} when sufficient time was allowed to elapse since operation for the atrophy of the submaxillary glands to attain over 20%. Castration does not reduce the concentration of I^{131} by the submaxillary gland in females.

The administration of testosterone propionate greatly increased the concentration of I^{131} by the submaxillary glands in castrated mice. However, the administration of estradiol benzoate and dipropionate did not alter this concentration.

There is an evident parallelism between the height of the epithelium of the alveolar ducts and the concentration of I^{131} by the submaxillary glands, which would support the hypothesis of the role of this epithelium in the concentration of I^{131}.

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SOME FEATURES OF THE ABSTINENCE SYNDROME TO MORPHINE IN MICE

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It has been postulated that morphine administered as a pellet is well tolerated in the white mouse, and produces all the typical signs of morphinism. Besides, it induces tolerance easily, and after some days of the implantation a dose of nalorphine develops the peculiar "abstinence syndrome" ([11]). The abstinence syndrome presents many symptoms which are intense and easy to reproduce in white mice. Because of these characteristics it was thought of interest to study some aspects of it in order to have a better knowledge of its mechanism of action.

Method

Adult male and female white mice (Catholic University's strain) were used. In some cases, the DBA mouse (deep brown) was used. (*) Special care was taken to use animals of the same weight. Mice were divided in many groups to carry on the experiments. Each group had 6 or more animals.

Drugs employed were: a) Morphine hydrochloride (Ingelheim) transformed to the base, washed with water and used without further chemical purification. It was compressed as pellets without other substances and implanted aseptically in the cellular tissue of the back of the animals. Knowing the absorption rate (the pellets take 30 to 32 days to be absorbed ([11]), as well as the weight of the pellet and the weight of the animal, it was possible to estimate the daily absorption dose of the drug by kilogram of mouse. When pellets of morphine were employed, the dose was always refered to as miligrams absorbed per day and per kilogram of animal. b) Morphine hydrochloride dissolved in water at the doses indicated under "results". c) Nalorphine (Allylnormorphine) in water solution at the dose of 10 mg per kilogram. Drug solutions were injected in the anterior wall of the chest.

Results

1. — Time necessary to develop the abstinence syndrome. — Two or 3 minutes after the administration of nalorphine to mice implanted with morphine, the abstinence syndrome appeared. Its characteristics are described elsewhere ([11]). In order to study how

(*) We thank Dr. G. Hoecker for the DBA mice supplied.

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soon the syndrome appeared a pellet of morphine was administered in such a dose that an intense abstinence syndrome could be expected. Two groups of mice were separated. One absorbed 308 mg per kilogram and per day and the other one, 600 mg (it was not possible to exceed this amount because in one implant of 600 mg a high mortality was observed). These animals were injected with nalorphine (10 mg per kilogram) 12, 24, 36 and 48 hours after the implantation.

Independently from the dose of morphine, the abstinence syndrome was certainly recognized 24 hours after morphine. Animals presented hypermotility and jumping; they threw themselves to the ground and dyspnea appeared; some ones presented the "tail reaction". It is very difficult to indicate if nalorphine induced the syndrome or not 12 hours after the implantation of the pellets; its signs were confused, partially similar to the controls though a slight hypermotility seemed to be present in some mice. Thirty six and fourty eight hours after the morphine implantation the syndrome was firmly constituted; symptoms were intense, prolonged and abundant. Soft or liquid feces, tear secretion, micturition, etc., generally appeared after 48 hours.

Dark brown animals were also used and divided into two groups. The daily absorbed morphine was calculated to be 270 and 540 mg per kilogram of weight. Nalorphine was also administered 12, 24, 36 and 48 hours after morphine. As in the case of white mice it is not possible to ascertain if the abstinence syndrome appeared 12 hours after the implantation. At 24 hours, exaggerated hypermotility was observed and the "tail reaction" became positive. Thirty six hours after implantation of the pellets, animals were excited; they ran and threw themselves to the ground; tremor and dyspnea were seen in some ones, and in others soft or liquid feces were present. At 48 hours symptomatology was intensified.

2. — Doses of morphine administered as pellet and the abstinence syndrome. — When a small quantity of morphine is implanted in the cellular tissue of the mouse, the animal tolerates the drug very well. However if the implanted dose increases, besides the more pronounced intensity of morphinic symptoms toxic reaction appears and kills some animals. The larger the dose, the greater the death incidence. With implants that represent more than 500 to 600 mg of morphine absorbed per day and per kilogram, the percentage of survival is relatively low. The animal becomes sleepy, does not eat nor drink water, looses weight (20 to 25%) and dies. The second day is the worst; nevertheless, some mice die 3 or 4 days after the implantation. The autopsy does not reveal any anormality; thus, death must be attributed to morphine. If a great deposit of morphine is wished, it is necessary to implant pellets in various occassions in order to avoid a large number of deaths. After the second day, the probability to live increases considerably; mice gain weight and morphinism vanishes little by little. On the other hand, the lower the dose of the drug is, the sooner the signs of morphinism disappear.

Many experiments were performed to induce the abstinence syndrome. Two variables were considered in order to learn better the phenomenon: a) Dose: Nalorphine was administered at a given time after the implantation to groups with different doses of morphine; b) Time: a same dose of morphine was employed, but the syndromes were evoked at different times. For a better understanding of the general course of the experiments, the scheme of all the experiences performed in this section are represented in fig. 1.

It is necessary to remember and to emphasize that the abstinence syndrome

never presents all its symptoms with the same characteristics in the animals. On the contrary, symptoms vary in intensity, frequency and quantity as it was stressed (11). For these reasons it is not possible to make a very fine discrimi-

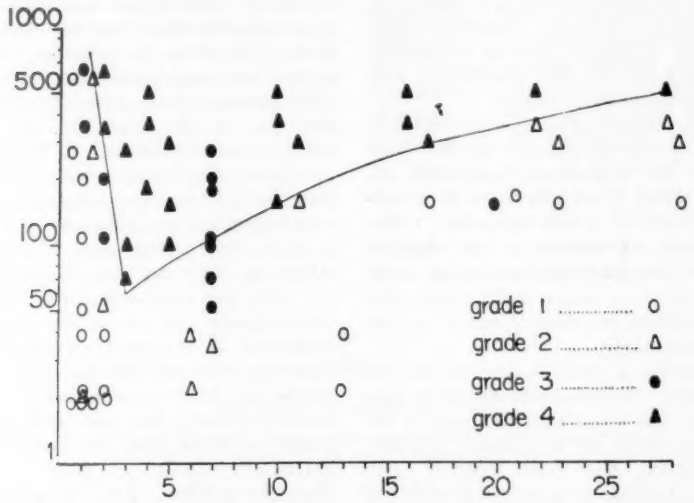

FIG. 1. — *Intensity of the abstinence syndrome and relationship between doses of morphine and time in which the syndrome is provoked.* Ordinates: *dose of morphine in mg.* Abscissa: *time in days (for further explanation, see text).*

natory evaluation of it in order to get very precise comparisons. The difficulty increases if it is wished to compare syndromes separated by long periods; the investigator's notes lose value, because many important details may pass off, and the subjective impressions are forgotten. Nevertheless, a very rough appreciation of the whole picture is of interest. According to this, and if the signs are simplified and grouped together, five different intensities (grades) of the syndrome may be separated:

First grade: animals become restless and smell in diverse directions.

Second grade: Animals occasionally run or jump; sometimes they throw themselves to the ground.

Third grade. Running and jumping are frequent; neurological signs are weak.

Fourth grade: Running and jumping become important; neurological symptoms are evident; "tail reaction" is positive.

Fifth grade: All described symptoms (in grade 4) are very intense.

Soft or liquid feces and dyspnea are not considered because they may be present in all grades; their intensity may vary.

In figure 1 each grade of intensity is represented by a symbol. Through the study of the figure, the following may be obtained:

1) Whatever the dose absorbed per day and per kilogram of animal may be, the time of appearence of the

abstinence syndrome is always the same: it starts approximately 12 to 24 hours after the morphine implantation.

2) The intensity of the abstinence syndrome increases with time, little by little when the dose of morphine is small, and more rapidly when the quantity is large. After reaching a maximum, the intensity decreases. However, when the daily absorbed dose is important, the intensity of the abstinence syndrome ressembles a plateau.

3) The larger the daily absorbed dose per kilogram of animal is, the more intense the abstinence syndrome obtained. Over a certain limit it is very difficult to get more intensity. When the pellets of morphine are administered in several opportunities, in order to get an even larger daily absorption it is possible to observe more violent symptomatology.

If points 2 and 3 are considered altogether, and represented in a graphic, as it is illustrated in figure 1 the peculiar shape of a triangle appears, delimitating the most intense syndromes. Its base is in relation with the larger doses and the apex, with the smaller ones; the more perpendicular side is dealing with time near the implantation and the more horizontal border, far from it.

Besides the already indicated peculiarities it is possible to add the following considerations: a) a minimal quantity of morphine absorbed per day and per kilogram was necessary to induce the syndrome. With a daily absorption inferior to 15 mg it was not possible to observe it, no matter when nalorphine was injected; b) it was thought of importance to explore if the intensity of the abstinence syndrome had some relationship with the total dose, independently from the time taken to absorb a definite quantity of morphine, i. e. if the intensity of the syndrome was the same in two groups of animals, the first one having absorbed a quantity of the drug in 3 days and the second one in 15 days for instance. No correlation was found between the intensity and the total dose of the drug; c) as it has been described the abstinence syndrome has 2 different periods. The first one corresponds to the phase of hypermotility, which is followed by a second period in which immobility predominates. The first phase lasts 30 minutes, and the second one, approximately 1 hour ([11]). The present study indicates that the duration of the hypermotility period tends to become longer if the dose of morphine implanted at once is large, but there is no mathematical correlation between these phenomena.

With a very large dose of morphine (1000 to 2000 mg per kilogram and per day) the temporal course and the characteristic picture of the abstinence syndrome is somewhat different: the intensity does not increase in an appreciable way (only in some cases it attains much intensity) but the hypermotility phase prolongs, and can last 1½ to 2 hours; afterwards, symptoms of morphinism are evident. This morphinism is more intense the larger the dose of implanted morphine is. In the case of a daily absorption of 2000 mg, a not very intense period of hypermotility has been observed (animals are continuously depressed) but neurological signs are predominant and a special and notorious alteration of the muscular tone (hypotonia) is seen.

3. — Doses of morphine administered in solution and the abstinence syndrome. — It has been claimed that it is necessary to administer frequent large doses of morphine to rats, during a long time, in order to induce an intense abstinence syndrome, because if the doses are small and separated by long intervals, nalorphine does not produce the picture ([7]). This contrasts with the facts observed in mice. As it was emphasized in the anterior section, mice implanted with pellets of morphine can

already evoke an abstinence syndrome if nalorphine is injected 24 hours after the administration of a relatively weak dose of the natural alkaloid. In order to elucidate if the difference was due to the diverse species studied (rats and mice) or to the morphine administration techniques employed, 5 groups of animals were used, and different experiments were performed. Morphine was injected at different intervals and at diverse doses. Results were always compared with those observed with the same quantity of daily doses of morphine absorbed from pellets.

Results, summarized in table I show that the more frequent the administration of morphine is, the more intense and easy to obtain is the abstinence syndrome. Thus, 80 mg per kilogram of weight injected twice a day do not induce the syndrome after 12 days of treatment. However, the same daily quantity administered in doses of 40 mg per kilogram every 4 hours (the drug was not injected during the night) produces the picture at the fourth day and its intensity increases during the following 3 days. When smaller doses are employed, injected every 4 hours, an abstinence syndrome of lesser intensity is obtained. No significant differences in intensity are observed when morphine is employed in increasing schedules. Notwithstanding, it is possible to demonstrate an abstinence syndrome 3 days after the beginning of the treat-

TABLE I

Scheme of morphine injections

	DAY	1	2	3	4	5	6	7	8	TOTAL DOSE mg
					INJECTIONS OF MORPHINE					
maintained dose		80/2	80/2	80/2	80/2 (?)	80/2	80/2	80/2 (?)	80/2	1280
		40/4	40/4	40/4	40/4 (1.5)	40/4	40/4	40/4 (2)	40/4	1280
		10/4	10/4	10/4	10/4	10/4	10/4	10/4	10/4 (1)	640
increasing dose		10/4	25/4	40/4	50/4	60/4	60/4	80/4	100/4 (1.5)	1700
		50/4	60/4	60/4 (1.5)	80/4	100/4	120/4 (2)			1880
					PELLETS OF MORPHINE					
daily equivalent dose (in mg)				240 (3.5)	160 (4)		480 (4)	160 (3)	40 (2.5) 400 (4)	

Left to the separation line: doses of morphine in mg; right to the separation line: number of injections per day. In brackets: appreciation of intensity grade; (?) doubtful interpretation.

ment, when morphine is injected every 4 hours in increasing doses.

Comparing what happens when morphine is administered as injection or as pellet, one is impressed by the severity attained by the abstinence syndrome in this last case.

In order to control the symptoms of the abstinence syndrome doses of morphine up to 100 mg per kilogram of weight were injected to mice. Any abnormality produced by the drug was similar to that observed in the abstinence syndrome.

4. — Effects of the extirpation of pellets of morphine. — Pellets were extirpated under ether anesthesia. Many groups of mice implanted with different doses of morphine were studied. However, special care was taken with a lot of animals that had been implanted 26 days before and at the moment of the pellets extraction were absorbing 476 mg of the drug per kg and per day. They were followed until 24 hours after the anesthesia recuperation. During this interval, a very diffuse and weak symptomatology could be appreciated. Extirpation of pellets of lower doses, or suppression of the injections did not induce any symptoms.

Discussion

The abstinence syndrome is observed in mice only if nalorphine is employed to induce it. It does not appear when the pellets of morphine are extirpated or when the injection treatment is discontinued. Thus, nalorphine seems to be indispensable to detect the dependence, at least in small animals.

The white mouse seems to be a specially indicated animal to study the abstinence syndrome induced by nalorphine. Actually, its symptomatology is varied and intense, and it may be produced at will even by a low dose of the drug. If the compound is administered as a pellet, it is possible to evoke it 12 to 24 hours after the implantation. Morphine administered through the classic method of injection produces also an early syndrome, provided the substance is injected frequently (every 4 hours). Thus it appears that the frequency of administration is of greater importance in inducing the syndrome, than the period during which the product has been provided.

It was emphasized (see "results") how dificult it is to appraise the abstinence syndrome, because of its multiple and changing symptomatology that modifies the frequency, intensity, etc. of the signs from one animal to another, and because it is impossible to indicate the value of one symptom in comparison with another. Furthermore, the difficulty increases due to the resting period seen when hypermotility is over; its passage to normality as it is obvious, is hard to evaluate. These facts, in agreement with those indicated by Carter and Wikler ([1]), makes any comparison very rough and any fine estimation is lost. Besides, the evaluation is based on subjective appreciations, whose details must be memorized and retained to permit comparisons later on. In spite of all, it has been essential to clasify the abstinence syndrome in order to better understanding. If signs are grouped together and if small details are not considered, a useful classification may be obtained. Thus the five grades of the abstinence syndrome (dealing with its intensity) were born.

This simple classification allows to learn some important facts:

1) A minimum quantity of morphine and a minimum time are needed in order to induce the functional disturbances of the syndrome. As it was stressed, morphine administered as pellets needs one day in order to induce the syndrome. On the other hand, it is necessary to remember that when the narcotic is injected every 4 hours, the syndrome is observed for the first time between the third and seventh day af-

ter starting the treatment. These facts are the exponent of the great value of the pellet method and the sensitivity of the white mice for demonstrating the picture.

2) At a given time, the larger the dose of morphine is, the greater the intensity of the syndrome, until a limit is reached which is difficult to trespass. Dealing with this point, the following facts need some discussion: there would be a progressive deposit of morphine in the zones of the nervous system —responsible for the syndrome— until to arrive to its saturation. A larger quantity of the drug would not be employed for this purpose, and from this point of view the difference —a luxury dose— would be probably used for other activities. This supposition cannot be clearly supported by the present experiments and knowledge. The abstinence syndrome is a complex picture in which many non clearly understood factors intervene: a) morphine; its action, the establishment of tolerance to it or the combination of both; b) the own action of nalorphine; c) the suppression of the morphine effect; d) the vanishing effect of nalorphine and e) the quickly functional readaptation to the action of morphine. It has been observed that the larger the dose of morphine implanted, the greater the probability to observe signs of morphinism at the end of the abstinence syndrome induced by 10 mg of nalorphine. On the other hand, if high doses of nalorphine (100 mg per kilogram of weight) are employed to evoke the picture, it is only possible to observe the syndrome in those cases of large morphine implantation; when doses are smaller, the syndrome is hidden in the effects of nalorphine ([8]).

It would be interesting to discuss the interrelationships of the abstinence syndrome (expression of the physical dependence) and tolerance. It has been indicated that when mice receive a large dose of morphine, animals can die 2 to 4 days afterwards. If morphine kills the mice, it is evident that marked tolerance has not been developed yet. Nevertheless, the physical dependence is solidly established because at this time, one observes an intense abstinence syndrome. On the other hand, the easily observable morphinic signs (Straub reaction, exophthalmus, general behaviour of the mouse) take more and more time to disappear (without an appreciable limit) the larger the dose of administered morphine is. It must be remembered that the abstinence syndrome in its increasing intensity has a limit, beyond which there is a decaying period. This would be another argument against the possibility that morphine may use the same receptors to induce both phenomena.

3) According to the time elapsed the syndrome increases in intensity arriving to a limit which can be a plateau if a large quantity of morphine is implanted. Afterwards, the intensity of the syndrome decays (fig. 1). This is a very interesting phenomenon, not related with an increasing perturbation of the pellet absorption ([11]); there is no evidence neither of the substitution of the morphine of the pellet by important quantity of inert material (ghost formation ([5, 4, 3]). The study of microscopic sections is subject to discussion (it reveals a diffuse reticular formation of an albuminoid material), but the appraisal of differences of weight of the protein found in the pellet is not significant: in 12 days there is an increase of 1% of weight. Perphaps, the loss of intensity of the syndrome deals with an organic defensive mechanism to morphine, not yet defined.

There is not an adequate hypothesis to explain the abstinence syndrome ([9]). If one imagines that it is produced by a rapid suppression of the morphine activity in the central nervous system, it is logic to suppose that the whole nervous system participates in the pro-

duction of it. In behalf of the idea, some authors ([6, 14]) indicate that the drug may act along the total nervous axis and practically it is possible to detect it with labeled C14 drug in all its structures ([12, 13]). Several papers confirm this through sections of the nervous system at different levels ([14]). A pharmacological approach would be of interest. The fact that practically all the central nervous system intervenes, that it is produced in a very large number of zoological species ([9, 14]) and that its symptomatology is produced in such a short time after nalorphine, may be an index that morphine may incorporate itself actively, in some essential metabolic chain; after a short time (if it is administered continuously in adequate dose) its presence may be so important that its violent suppression could signify a deep and fundamental perturbation of the new metabolic arrangement.

The mechanism of action of nalorphine as antagonistic substance to morphine is not perfectly understood ([15]). However, the similar chemical structure of both would permit a chemical competition to the morphine receptor and displace explosively the natural alkaloid from the metabolic chain. If nalorphine is administered in adequate dose, the statistical probability to displace morphine is large; it must be remembered that nalorphine counteracts tachyphylaxis "in vitro" ([10]) (to understand tachyphylaxis and the significance herewith discussed, see 2). When nalorphine is not employed, or when the morphine pellets are extirpated or injections of morphine are discontinued, the morphine elimination from the hypothetical metabolic chain would be slow and the statistical possibility to be displaced simultaneously or in a short time from all the basic receptors would be negligible. In this way, a compensatory mechanism would be set forth.

SUMMARY

The abstinence syndrome to morphine was induced in white mice implanted with pellets by the action of nalorphine (Allylnormorphine). In order to control the drug method of administration, regular injections of the alkaloid were performed. The following facts could be deduced:

1) It is necessary a minimum of time of implantation (12-24 hours) and a minimum dose of morphine (over 15 mg absorbed per kilogram of animal and per day) in order to induce the syndrome.

2) A tentative classification of the syndrome intensity is described: it may be emphasized that: a) The time of appearance of the syndrome is independent of the morphine dose; b) For a given dose, the intensity of the syndrome increases with time until to reach a limit after which the intensity decays; c) there is a direct relationship between the intensity of the syndrome and the daily absorbed dose of morphine; when the abstinence syndrome reaches a limit, it is very difficult to trespass it.

3) There is no relationship between the intensity of the syndrome and the total dose of morphine, whatever the time to absorb it may be.

4) The species (white mice) and the pellet method of morphine administration are two very important facts to induce easily the syndrome, with relatively low doses of the drug. Pellet administration is a better method than the regular injection of the drug.

5) Nalorphine seems to be necessary to induce the syndrome. It is not evoked in the white mice by the extirpation of the pellet nor when regular injections are discontinued.

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ESTERILIDAD EXPERIMENTAL PROLONGADA EN RATONES Y RECUPERACIÓN DE LA FERTILIDAD (*)

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El moderno problema de la inducción experimental de esterilidad por la administración de esteroides, como ha sido concebido por Pincus, está íntimamente ligado con la función gonadotrófica de la hipófisis.

Es de conocimiento general que la función gonadotrófica de la hipófisis está, por su parte, bajo la dependencia de las hormonas gonadales. Ha sido estudiada, en especial, la influencia que los estrógenos ejercen sobre esta función gonadotrófica. Pero también la influencia de la *progesterona* adquirió interés preponderante (Dempsey y otr., 1936); Dempsey, 1937; Selye y otr., 1936); Selye, 1939; Makepiece y otr., 1936, 1937). Estos datos experimentales han sido resumidos repetidamente en el curso de los años (Burrows, 1949, cap. XXII; Courrier, 1950).

Un impulso para nuevos estudios experimentales con progesterona y derivados progestativos nos dió, hace años, el hallazgo que en el ovario injertado en el bazo, en ausencia del segundo ovario, se produce un desvío del desarrollo folicular: aparecen folículos hemorrágicos (Lipschutz y Ponce de León y otr., 1944, 1946; resúmenes: Lipschutz, 1956, 1958). Era evidente que en las mencionadas condiciones experimentales existe una disfunción hipofisaria gonadotrófica. Esta se explica por la inactivación parcial que sufren los estrógenos al pasar del ovario intraesplénico primero por el hígado, antes de llegar a la circulación general. Al administrar estrógeno por vía subcutánea no se presentan los folículos hemorrágicos en el injerto intraesplénico. Pero, al administrar cantidades relativamente grandes de estrógeno se estimula la luteinización.

El injerto ovárico intraesplénico ofrece la oportunidad de estudiar la influencia de los esteroides y, en especial, de la progesterona sobre la función gonadotrófica de la hipófisis, con mayor facilidad que el ovario *in situ* ya que en nuestras condiciones experimentales, el eje ovario-hipófisis es, hasta cierto grado, liberado de la interferencia de los esteroides ováricos producidos por el ovario mismo.

Al administrar progesterona por vía subcutánea, la luteinización inducida en el injerto ovárico intraesplénico por

(*) Conferencia dictada en la IV Reunión de la Asociación Latinoamericana de Ciencias Fisiológicas, Ribeirão Prêto, São Paulo, Brasil, 4-8 julio, 1961.

CUADRO Nº 1

25 esteroides, naturales y sintéticos, cuya acción antiluteinizante ha sido estudiada

1 progesterona (P)
2 Δ^{11}-dehidro-P
3 19-nor-P
4 9α-F-11β-OH-P
5 9α-Br-11-keto-P
6 Δ^{6}-dehidro-6α-Cl-17α-acetoxi-P
7 nor-A-P
8 21-OH-P (desoxicorticosterona)
9 11β-OH-P
10 11α-OH-P
11 11-keto-P
12 17α-OH-P
13 17α-OH-P, capr.
14 17α-OH-P, acet.
15 21,17α-di-OH-P, acet. (S)
16 21,11β,17α-tri-OH-P (hidrocortisona)
17 21,11β,17α-tri-OH-P, acet.
18 2-metil-hidrocortisona
19 9α-F-metilhidrocortisona
20 cortisona, acet.
21 vinil-testosterona
22 etinil-testosterona
23 19-nor-etinil-testosterona
24 etinil-Δ^{5}-androstendiol
25 19-nor-metalil-testosterona

el estrógeno, ya no se produce. La progesterona actúa de *antiluteinizante* (Lipschutz, Iglesias y otr., 1948; Iglesias y otr., 1948, 1950; Mardones y otr., 1951; Mardones, Iglesias y Lipschutz, 1954a; Lipschutz, Jadrijevic y otr., 1957; Figueroa y Lipschutz, 1956, 1957)

En el curso de los años hemos estudiado de modo comparativo la acción antiluteinizante de más de 20 diferentes esteroides. Nos encontramos con varios aspectos químicos de gran interés, pero que el tiempo no nos permite discutir aquí. Las sustancias más activas son progesteronas sintéticas, en especial la 19-nor progesterona (Mardones, Iglesias y Lipschutz, 1954b, 1956) y el fluoro-derivado de la progesterona (Lipschutz y Figueroa, 1957). (Véase Cuadro 1 a 4).

CUADRO Nº 2

Acción antiluteinizante de la progesterona (P) y de otros esteroides. Cob., inj. ovár. intraesplén.: 30 a 60 µg de estradiol/día.

Esteroide	µg/día	Índice de luteiniz.
0	0	2.4
P	12	1.5
P	20	0.7
P	40	0
11β-OH-P	47	0.2
11α-OH-P	43	2.1
21-OH-P	200-400	0.4
17α-OH-P	130-287	2.0
Hidrocort.	5140 (!)	1.4
Cortisona	1742 (!)	2.4

CUADRO Nº 3

Acción antiluteinizante comparativa de P y 19-nor-P. Cob.; inj. ovár. intraesplén.

Esteroide	µg/día	Índice de luteiniz.
0	0	2.4
P	12	1.5
P	20	0.7
P	40	0
19-nor-P	3	0.05
19-nor-P	19	0

La acción antiluteinizante de 19-nor-P es m/m 10 veces mayor que de P.

CUADRO Nº 4

Acción antiluteiniz. de varios esteroides. Cob.; inj. intraesplén.

Esteroide	μg/día	Indice luteiniz.
9α-F-11β-OH-P	3	0.6
9α-F-11β-OH-P	10	0
Etinil-testost.	185	2.0
19-nor-etinil-testoster.	113	0.5

No se trata de una acción local de la progesterona sobre el ovario. Al implantar un comprimido de progesterona en el ovario mismo y éste en el bazo, no se presenta la acción antiluteinizante (Iglesias y Rojas; véase Rojas, 1949). Debe tratarse de una acción dirigida hacia la hipófisis o el hipotálamo. Para simplificar, hablamos de una acción *hipofisótropa*.

Una cuestión de sumo interés se presenta aquí: las cantidades antiluteinizantes de progesterona que hemos usado en nuestra experimentación, ¿están todavía en el marco fisiológico o lo sobrepasan? Discutimos esta cuestión en ocasiones anteriores y llegamos a la conclusión que estas cantidades antiluteinizantes experimentales de la progesterona están todavía muy en el marco de la normalidad fisiológica.

En nuestros experimentos, 20 a 30 μg por día absorbidos desde un comprimido subcutáneo de progesterona eran suficientes para inhibir la luteinización inducida por el estrógeno. ¿Qué cantidad de progesterona produce por día el ovario? No conocemos la concentración de progesterona en la sangre del cobayo pero sí en otras especies incluso en la mujer (trabajos anteriores de Hooker y Forbes, 1947, 1949; nuevos trabajos de gran alcance, en la mujer, yegua, vaca, oveja, cerda y cabra, de Short, 1960). Un cobayo de 500 a 700 g tiene alrededor de 36 a 50 cc de sangre (Ancill, 1956). Short admite para la cobaya, en la fase luteica, un contenido de progesterona de 0.5 μg por 100 cc de sangre. Así estarían presentes en la sangre circulante de la cobaya en todo momento hasta 0.25 μg de progesterona. Short calcula con la necesidad de renovar esta cantidad cada 10 minutos. Esto significaría una producción de progesterona al nivel de ovario, de hasta 36 μg por día. Es de gran interés que esta cantidad está muy cerca de aquella, la cual en nuestra experimentación se ha revelado ser suficiente para controlar la función gonadotrófica luteinizante de la hipórfisis estimulada por el estrógeno. En cuanto a la producción de la progesterona en los dos ovarios del cobayo, podemos hacer el siguiente cálcu-

CUADRO Nº 5

PROGESTERONA EN LA SANGRE DE LA COBAYA *

Volumen de la sangre del cobayo:	7.2cc/100g (Ancill) 36-50cc/500-700g
P en la sangre:	0.5 μg/100cc. 0.18-0.25μg/36-50cc.
Para mantener tal concentración, el ovario debe producir:	0.18-0.25μg/10 min. 1.1 -1.5 μg/hora 26 -36 μg/día (Short)
P en 1 C. lut. de cob.	0.05μg/ (Rowlands & Short)
P en 3 C. lut. (promedio)	0.15μg

* Cálculo gentilmente hecho para nosotros por el Dr. R. V. Short (1961) basándose en sus amplios estudios sobre la progesterona en la sangre. Debemos al Profesor R. Caldeyro-Barcia de haber llamado nuestra atención, en la discusión de nuestra conferencia, a los trabajos de Short.

lo. Un cuerpo lúteo de la cobaya no preñada contiene 0.05 μg de progesterona (Rowlands y Short, 1959); en los tres cuerpos lúteos que son el promedio en cada ciclo ovárico, habrá así 0.15 μg. De este modo los cuerpos lúteos deberían renovar cada 5 a 10 minutos toda la cantidad de progesterona presente en ellos. Uno fácilmente se da cuenta de la intensa labor sintética no interrumpida que debe ser realizada en el seno de los cuerpos lúteos para suplir a las exigencias del organismo en progesterona.

Es del todo evidente que con nuestro modo de trabajar en el problema de la acción antiluteinizante de la progesterona estábamos en el marco de la normalidad cuantitativa. Pero, por otra parte, uno se preguntará si lo que vale para el injerto ovárico intraesplénico, valdrá también para el ovario *in situ*. Los autores que nos precedieron en los estudios relativos a la acción de los esteroides sobre el eje ovario-hipófisis, han trabajado con el ovario *in situ* en el cobayo (Dempsey y otr., 1936; Dempsey, 1937), en el conejo (Makespiece y otr., 1936, 1937), en la rata y en el ratón (Selye y otr., 1936; Selye, 1939). Más recientemente la acción antiluteinizante de la progesterona sobre el ovario *in situ* en la cobaya fué comprobada por la Dra. Panasewich (1960). (Véase Cuadro 6.)

CUADRO Nº 6

Acción antiluteinizante de P sobre ovario "in situ". Cobayas, 430 a 600 g. P: 270 a 468 μg/día. Exp. de Dra. Panasewich

Grupo	Nº de anim.	Nº de ovarios exam.	Nº de ovarios con C. l.
Norm.	15	30	28
Con P	20	40	0

De gran interés han sido los estudios experimentales en la rata. Puede haber amplia luteinización aún al administrar progesterona (Selye y Friedman, 1940; Rothchild, 1960; Iglesias, 1960). Sin embargo, un estudio más detallado ha revelado que la extensión de la luteinización es, en animales con progesterona, considerablemente menor que en animales de control sin progesterona (para la técnica aplicada, véase Lipschutz, 1933). (Cuadro 7.)

CUADRO Nº 7

Acción antiluteotrófica de P, sobre ovario "in situ". Ratas, 90 a 100 g. P 367 a 460 μg/día. Experimento de Iglesias

Grupo	Nº de anim.	Nº de ovar. exam.	Ovario prom. mg	Luteiniz. %	C. l. mg
Norm.	10	15	29	47.8	13.9 (100)
Con P	8 *	16	23	40.5	9.3 (67)

* 1 animal sin C. l., fuera de los 8.

Estas observaciones evidencian que la acción hipofisótropa antiluteinizante de la progesterona es un fenómeno más complejo que lo que se pensaba anteriormente. Por una parte, los experimentos en el cobayo, y así también en el ratón, no dejan duda que la progesterona inhibe en estas especies la entrega de la *hormona luteinizante* (LH) estando ausentes los cuerpos lúteos cuando se administra progesterona. Pero, por otra parte, los nuevos hallazgos de Iglesias en la rata hacen pensar que aún al no ser inhibida la producción de cuerpos lúteos por la progesterona, influye ella en alguna forma sobre la evolución ulterior del cuerpo lúteo. El número promedio de cuerpos lúteos por ovario bajó de 10 a 8, es decir en 20 por ciento, pero el peso total de los cuerpos lúteos bajó en 37 por ciento. Por eso tenemos que suponer que la progesterona controla también la entrega de la hormona *luteotrófica* (LtH) de la hipófisis.

Quien se compenetra de los resultados sobre la acción antiluteinizante comparativa de la progesterona en diversas especies y, en especial, en el cobayo y la rata, se da cuenta de lo atractivo que es el problema, y de su importancia para la comprensión de las intrincadas relaciones hormonales en las cuales se basan la secuencia de las fases en el ritmo sexual de los mamíferos e incluso en el ritmo sexual de la mujer, fenómeno complejo y que hasta ahora continúa muy lejos de ser dilucidado.

Es como ya mencionamos, el gran mérito de Pincus y de su grupo de haber concebido la idea de hacer uso de los esteroides para los fines del control de la reproducción. La hormona del cuerpo lúteo tiene acción antiovulatoria. Hace 32 años Parkes (1929) en su libro sobre "La Secreción Interna del Ovario", escribió un capítulo dedicado a la inhibición de la ovulación por el cuerpo lúteo persistente. Este capítulo basado enteramente en la experimentación clásica de Loeb, de Hammond, y de otros, conserva hasta hoy su actualidad. No podemos entrar en la discusión de los problemas especiales que aquí se presentan y que fueron estudiados por Pincus y col. en extensos trabajos experimentales (Pincus y Chang, 1953; Pincus y otr., 1956, 1956). Atengámonos sólo al hecho que la acción antiluteinizante de la progesterona conduce implícitamente a la esterilidad; y el grupo de Pincus ha conseguido así controlar la concepción en la mujer al administrar esteroides progestativos que actúan por boca, como la etinil-19-nortestosterona u otros (Rock, García y Pincus, 1960). El esteroide se da al quinto día después de la última menstrucción, durante veinte días. Al cesar el tratamiento se presenta la menstruación. Los autores han conseguido mantener así a la mujer durante dos años y medio, sin que tuviera lugar una concepción y sin que se produzcan trastornos duraderos. En un estudio que abarca miles de ciclos sexuales en cientos de mujeres, el tratamiento anticoncepcional falló sólo en 1 por ciento de los ciclos.

Desde un punto de vista *clínico*, tuvo que plantearse la cuestión de si el tratamiento esterilizante prolongado puede significar un daño para el eje ovario-hipófisis, con infertilidad subsiguiente (Rock y otr., 1960). Las observaciones que los autores hicieron en mujeres después de cesar el tratamiento antiovulatorio no hablan en favor de algún daño consecutivo a tal tratamiento: en considerable número de mujeres hubo concepción después del cese del tratamiento antiovulatorio.

Sin embargo, la observación clínica no permite un amplio estudio de la cuestión de los daños inherentes a un prolongado tratamiento antiovulatorio. Hemos pensado que en cuanto a este problema, estudios experimentales en *ratones* ofrecerían una buena oportu-

nidad. Se plantean aquí los siguientes problemas especiales:

1) ¿Sería posible prolongar la esterilidad por administración de esteroides, por un tiempo que abarcara una considerable parte de la vida reproductora?

2) ¿Qué trastornos traería consigo tal esterilidad inducida y prolongada?

3) ¿Podrán los animales recuperar la fertilidad después de tal esterilidad inducida y prolongada?

4) ¿Cómo se presentará la fertilidad en sus condiciones cuantitativas, después de tal esterilidad inducida y prolongada?

Nuestros estudios se hicieron en la cepa BALB. La vida reproductora en esta cepa es bien conocida; ha sido estudiada en especial por el grupo de Mühlbock (Thung y otr., 1956; Mühlbock, 1957). En nuestra cepa la vida reproductora comienza a la edad de 2 meses. Pero unos 12 meses después la fertilidad expresada en el número de partos y en el número promedio de crías por parto comienza a disminuir, y los últimos 6 meses más o menos de vida la hembra normal los pasa sin reproducirse.

No sabemos cuáles son los factores responsables de esta secuencia de fases en la vida reproductora del mamífero. Así parece evidente que experimentos en los cuales una esterilidad es inducida por la acción de esteroides a los dos meses, para ser prolongada por largos meses, abrirían posibilidades antes ni siquiera sospechadas, para el estudio de los problemas referentes a los aspectos cronológicos y cuantitativos de la vida reproductora de los mamíferos.

Nos servimos en nuestros experimentos de uno de los más potentes antiluteinizantes, la 19-nor-progesterona (19-nor-P); a ella hay que dar preferencia ante los derivados halogenados porque nos convencimos que estos últimos no son inocuos para el ratón. La 19-nor-P fué administrada bajo forma de comprimidos implantados subcutáneamente. Los comprimidos contienen 20 o 40 por ciento de 19-nor-P mezclada con 80 o 60 por ciento de colesterol, lo que permite variar la cantidad de 19-nor-P absorbida en unas cinco o seis veces. La ley cuantitativa a la cual obedece la absorción a partir de semejantes mezclas ha sido estudiada en trabajos anteriores

CUADRO Nº 8

Esterilidad inducida con 19-nor-P, prolongada hasta 6 meses. Recuperación de la fertilidad. Ratones BALB

Nº de anim.	Edad días	Duración de ester. días	19-nor-P µg/día	Primer parto de recup. días	Crías primer parto
7	52	108	3.1	18-45 *	7.4 (2-11)
7	45	108	13.4	22-42	8.3 (4-13)
8	52	176	2.4	20-40	6.3 (1-9)

* 1 animal; 86 días.

(véase Fuenzalida, 1950; Lipschutz y Fuenzalida, 1953).

En un experimento preliminar de sólo 15 días de duración pudimos establecer que con 4 a 9 μg de 19-nor-P se puede prevenir la luteinización, en animales de 79 días. No menos decisivos fueron los experimentos de larga duración. (Cuadro 8.)

Implantamos en 14 hembras de 40 a 54 días de edad, comprimidos de 20 ó 40 por ciento de 19-nor-P. Junto con las hembras siempre estaba presente en la misma jaula, el macho. No hubo preñez en ninguno de los 14 animales.

Al cumplirse 108 días de la acción de 19-nor-P, los comprimidos fueron retirados; la absorción del esteroide fué, en el promedio, de 3 y 13 μg por día. En el curso del primer mes después de retirar la 19-nor-P, se preñaron 13 de los 14 animales; hubo un animal que se demoró más de dos meses en preñarse.

Semejante atraso en la producción de la preñez se ha observado en ratones después de un tratamiento con 19-noretiniltestosterona prolongado durante sólo 32 días (Bruce, 1959). Pero es notable que al fin y al cabo todas nuestras hembras se habían preñado. El promedio de crías por parto era de 7.4 y 8.3; es decir los animales, después de pasar por un período de esterilidad de tres meses y medio, eran tan fértiles como animales normales de esta cepa. Hubo tres animales aún con un número máximo de crías por parto, es decir de 10, 11 y 13.

Las crías fueron amamantadas igual que por madres con antecedentes normales; del parto de 13 crías, 12 fueron amamantadas. Por su parte, las hembras de la nueva generación, de antecedentes maternos tan anormales, comenzaron a preñarse a la edad normal de 2 meses.

Los 108 días de esterilidad inducida representan un cuarto, o algo menos que un tercio, de la vida reproductora de esta cepa, hasta su normal y pronunciada declinación. En otras palabras, hubo completa recuperación de la fertilidad después de una esterilidad inducida por un esteroide antiluteinizante, y mantenida durante considerable parte de la vida reproductora. Los 108 días de esterilidad inducida en nuestros ratones corresponden a casi 7 años, ó 5 años a lo menos, en la vida reproductora de la mujer.

Este resultado —esperado, o no esperado, nos sería difícil de decirlo— nos indujo a realizar un experimento de mayor duración. Era un experimento de 176 días de esterilidad experimental.

El resultado ha sido igual al del experimento anterior. En el curso del primer mes después de retirar los comprimidos, todos los animales se habían preñado. El promedio de crías por parto era de 6.3, número normal para hembras de 8 a 9 meses de edad. Los 6 meses de esterilidad inducida corresponden a 12 años, ó 10 años a los menos, en la vida reproductora de la mujer. La coincidencia en el número de las crías en animales normales y transitoriamente estériles, es impresionante; tanto más que el número de crías por parto varía, para los unos y los otros, ¡entre 1 y 13!

Las cuatro cuestiones que se plantean cuando uno quiere atacar el problema de la esterilidad inducida y prolongada por una fracción considerable de la vida reproductora, se las puede contestar en la forma siguiente:

1) Sí, no ofrece dificultad alguna inducir con un esteroide antiluteinizante una esterilidad por un tiempo que abarca a lo menos la mitad de la vida reproductora.

2) El único trastorno que hasta ahora hemos observado es el atraso en la recuperación de la fertilidad; el atraso puede alcanzar excepcionalmente hasta más de dos meses.

3) La recuperación de la fertilidad es general; aún después de prolongar

la esterilidad inducida por la mitad de la vida reproductora. No hubo entre los 22 animales ni uno solo que no hubiera recuperado su fertilidad.

4) Ya en el primer parto el número de crías por parto alcanza a lo menos el número característico de esta cepa a la edad correspondiente.

Sin embargo, el problema cuantitativo reviste interés mucho mayor que lo pueden resumir estas palabras. Como lo hemos mencionado, los estudios que estamos realizando ofrecen también la oportunidad de abarcar experimentalmente diversos problemas fundamentales de la reproducción en los mamíferos. Daremos sólo un ejemplo.

Desde los clásicos experimentos de Hunter (1787) en la cerda, a fines del siglo XVIII, se sabe que queda inalterado el número de crías en los partos que tienen lugar después de la eliminación de uno de los dos ovarios. Los hallazgos de Hunter fueron comprobados en otros mamíferos, así por Hammond y Asdell en el conejo, por Hartman en el opossum, por nosotros en el cobayo (resumen en Lipschutz, 1927). El número de folículos que en el ciclo sexual de los mamíferos entran en desarrollo o que maduran, número que es característico para la especie, queda constante aun si se aumenta la reserva de ovocitos injertando un tercer ovario como lo hicimos hace muchos años en el cobayo. Así se hizo evidente que la función ovárica depende cuantitativamente no del ovario mismo, sino de algún factor extragonadal, como lo resume la llamada "ley de la constancia numérica folicular", formulada por uno de nosotros hace casi cuarenta años. Hoy sabemos que este factor extragonadal reside en el aparato hipofisario-hipotalámico.

Hunter encontró otro hecho de interés provocativo: la vida reproductora de la cerda semicastrada se acorta grandemente al comparar con la cerda normal. La cerda semicastrada entra prematuramente en reposo reproductor definitivo, después de haber dado vida a un número total de crías igual a sólo la *mitad* del número normal total de la cerda. ¿Por qué? Pensábamos que el ovario restante, al obedecer a la ley de la constancias numérica folicular, dictada por decirlo así desde arriba, llega antes del tiempo al *agotamiento de la reserva de ovocitos, o reserva folicular.* Tales condiciones ováricas cuantitativas, es decir prematuro agotamiento de óvulos, se han creado experimentalmente con la castración subtotal en la coneja (Lipschutz, 1928) y otros métodos (Jones y Krohn, 1960b). Parecía seguro que el fin de la vida reproductora en los mamíferos fuera dictado por una disminución, o el agotamiento, de la reserva en ovocitos en los ovarios. Esto parecía tanto más seguro ya que, de hecho, con la edad disminuye grandemente el número de ovocitos en los ovarios del animal normal, no importa si la hembra hubiera servido para la crianza, o se la hubiera mantenido virgen (Jones y Krohn, 1961; véase también 1961c).

Más recientemente Jones and Krohn (1960a), al estudiar la reproducción en ratones semicastrados, han confirmado de nuevo los hallazgos clásicos de Hunter: sí, el número de las crías en el parto alcanza en la hembra semicastrada el número característico de la hembra normal; pero el número de partos, y con eso el número total de crías, llega, igual que en la cerda, sólo a la mitad de lo normal. Los ratones normales con los dos ovarios intactos tuvieron su último parto a los 363 días; los animales con un solo ovario tuvieron su último parto ya a los 280 días. Sin embargo, cuando los mismos autores contaron los ovocitos en los ovarios de estas hembras semicastradas con fin prematuro de su vida reproductora, la reserva de ovocitos, o folículos primarios, *no* estaba agotada; el número de ovocitos todavía presentes no era menor que en ovarios normales. Estos resultados ha-

blan decididamente contra el concepto de que un agotamiento de los ovocitos fuera responsable del hecho de que en cierto momento de la vida comienza a declinar la función reproductora para cesar en seguida definitivamente.

En otras palabras: la declinación cuantitativa de la función ovárica del mamífero sería un fenómeno dependiente de condiciones *extra-ováricas*, relacionadas con la edad, o condiciones *gerontológicas* generales.

La esterilidad inducida por un esteroide y prolongada por tiempo tan considerable en nuestros experimentos representa, sin duda alguna, un formidable impacto sobre la hipófisis y el ovario, y con eso sobre la función reproductora en su conjunto dinámico. ¿Influiría tal impacto, muy prolongado aunque sólo transitorio, en la curva del descenso de la fertilidad?

Hemos estudiado la actividad reproductora de nuestros animales con recuperación de su fertilidad después de una esterilidad inducida por 19-nor-P durante muchos meses. Nos hemos convencido que después de la eliminación de los comprimidos, la curva de la fertilidad se presenta cuantitativamente como una réplica de la curva normal.

Nuestra curva de la fertilidad normal está basada en la anotación de 220 partos a diferentes edades, con un total de 1422 crías. La curva reproduce fielmente el hecho ya conocido de la disminución de la fertilidad en el ratón. Comparemos ahora la curva de los animales experimentales con la de los normales. (Fig. 1 y 2.)

A la edad de unos 14 meses se produjo la misma disminución del número de crías por parto como en hembras normales de la misma edad.

La tendencia hacia la disminución de la fertilidad se manifiesta con la misma claridad cuando se comparan los nueve partos que tuvieron lugar después de remover los comprimidos de los primeros 14 animales del Cuadro Nº 8

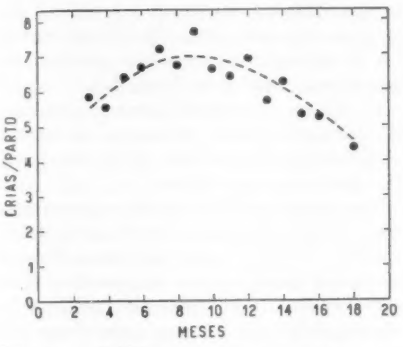

FIG. 1. *BALB: normales, 220 partos, 1422 crías.*

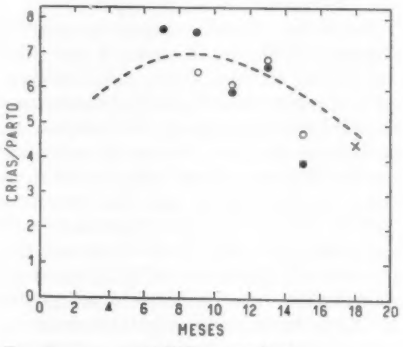

FIG. 2. — - - - - - - *BALB normales:*
•: *108 días de esterilidad inducida; en seguida 89 partos, 598 crías.*
○: *176 días de esterilidad inducida; en seguida 44 partos, 273 crías.*
x: *promedio más bajo en normales.*

(fig. 3). Es de interés también el hecho que a la edad de 10 meses una u otra hembra deja de tener parto. A la edad de 14 y 15 meses sólo tres y finalmente *una* de los catorce animales tiene parto.

Así, podemos decir que la inducción de esterilidad por la acción del esteroide antiluteinizante, esterilidad que puede ser prolongada por una gran parte de la vida reproductora, no cambia en nada el aspecto cuantitativo de la capacidad ovulatoria del ovario una vez que el esteroide estirilizante haya sido

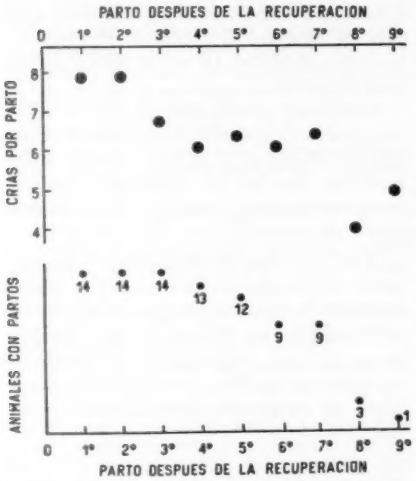
PARTO DESPUES DE LA RECUPERACION
0 1° 2° 3° 4° 5° 6° 7° 8° 9°
CRIAS POR PARTO
8
7
6
5
4
ANIMALES CON PARTOS
14 14 14 13 12 9 9 3 1
0 1° 2° 3° 4° 5° 6° 7° 8° 9°
PARTO DESPUES DE LA RECUPERACION

FIG. 3. — *Reproducción en 14 animales, después de 108 días de esterilidad inducida. Nueve partos.* Arriba: *crías por parto.* Abajo: *número de animales que tienen partos.*

reproducción después de la esterilidad inducida, por otra parte. La calidad reproductora de los animales experimentales coincide cuantitativamente con la del animal normal.

Es evidente que la esterilidad prolongada basada en el paro artificial de la función ovárica normal, no ha producido ningún cambio en la reproducción posterior a la eliminación del factor esterilizador. Tratándose en nuestros experimentos de un impacto verdaderamente tremendo en el eje ovario-hipófisis-hipotálamo, parece justo opinar que los resultados hablan de nuevo, y decididamente, en favor del concepto que la declinación y el cese de la función reproductora en la hembra del mamífero no depende del ovario, o del eje ovario-hipófisis-hipotálamo, sino de factores gerontológicos de otra localización o factores gerontológicos generales.

CUADRO Nº 9

Fertilidad comparativa: animales normales y animales con esterilidad inducida transitoria. El cuadro abarca 8 meses, desde el primer parto en el grupo de tres meses y medio.

Grupo	Duración de ester. induc. meses	Nº de partos	Nº de crías total	Nº de crías por parto
Norm.	0	140	927	6.6
Ester.	3½-6	133	871	6.5
(*) Ester.	3½	89	598	6.7
Ester.	6	44	273	6.2

(*) Los dos grupos de arriba calculados separadamente.

retirado: la intervención experimental no disminuye y tampoco aumenta esta capacidad.

En cuanto a eso, es muy convincente el cuadro 9 que resume la actividad reproductora comparativa durante ocho meses, de animales normales, por una parte, y de animales que volvieron a la

Por cierto, por el momento sería cosa muy atrevida pronunciarse en forma definitiva sobre este problema gerontológico de tanto interés. Tenemos ahora en marcha experimentos en los cuales la esterilidad inducida con 19-nor-P se prolongará por unos 14 meses, es decir hasta la fecha cuando la función re-

productora cesa en la hembra normal de nuestra cepa. Al retirar en esta misma fecha los comprimidos esterilizadores ¿tendrán *todavía* partos los animales que se mantuvieron estériles durante todo este tiempo?

Creo que no se podrá negar que el método experimental que preconizamos, con inducción de una esterilidad prolongada por la acción de 19-nor-P, ofrece nuevas posibilidades de atacar problemas fundamentales de la reproducción de los mamíferos.

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BIOQUÍMICA
BIOCHEMISTRY

The respiratory chain and the oxidative phosphorylation of rat brain mitochondria. M. BACILA, D. O. VOSS AND A. P. CAMPELLO. *(Inst. Bioquímica, Univ. do Paraná, Caixa Postal 939, Curitiba, Paraná, Brasil).*

Rat brain mitochondria was prepared according to the method described by Voss et *al.* (1). The oxidative phosphorylation was determined by the polarographic technique of Chance and Williams (2) using a polarograph described by Voss and Bacila (3). The differential spectra of the respiratory chain were determined at room and liquid nitrogen temperature by recording spectrophotometry. The *b* component was studied in a preparation containing succinate and then treated with antimycin *A*. At room temperature rat brain mitochondria showed the following peaks: 602 $m\mu$ (cyt. $a + a_3$), 562 $m\mu$ (cyt. b), 550 $m\mu$ (cyt. c), 518 $m\mu$ (cyt. $b + c$), 443 $m\mu$ (cyt. a_3), 429 $m\mu$ (cyt. b), and 419 $m\mu$ (cyt. c). At liquid nitrogen temperature the *b* component displayed two different peaks at 562 and 559 $m\mu$ when the antimycin-treated preparation was treated with dithionite. Also cytochrome c_1 showed a very clear peak at 554 $m\mu$. The relative concentration of cytochrome from rat brain mitochondria taken as equal to 1 was of 1.3 for cytochrome a_3, 0.6 for cytochrome b and 1.3 for cytochrome $c + c_1$. The ADP/O ratios for succinate and glutamate were 1.98, and 3.0 for α-ketoglutarate.

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Gyroxin, a new neurotoxin of *Crotalus durissus terrificus* venom. A. BARRIO. *(Inst. Nac. Microbiologia, Vélez Sársfield 563, Buenos Aires, Argentina).*

A new neurotoxin of the South American rattlesnake venom (*Crotalus durissus terrificus*) from Argentina and Southern Brazil has been identified and isolated by us, and because of its characteristic action on mice we have called it *Gyroxin*.

This substance has been separated from the crotoxin complex. By paper electrophoresis it can be located in the anodic front. For this purpose filter paper Whatman Nº 4; borate buffer pH 9.4 with ionic strength 0.18 were used.

Duration of run was 14 hours; current 10 volts/cm; venom solutions at 20-25 % in the same buffer.

Gyroxin can also be obtained by chromatography on Amberlite IRC-50 (XE-64). Columns of 4.5 cm diameter and 4 cm high were used with a flow rate of 18 ml/h. The resin was equilibrated with 0.1 M sodium phosphate buffer pH 6.4 and the column loaded with 100 mg of crotalic venom dissolved in the same buffer. The protein amounts of the eluates were followed by measuring the optical density at 280 $m\mu$. With this buffer we obtained first the paralysing neurotoxin (crotactin). When the optical density values indicated that no further protein was being eluted, the buffer was changed to 0.2 M disodium phosphate and 0.2 M NaCl, and a second peak emerged which corresponded to *Gyroxin*.

Finally augmenting the NaCl concentration to 2 M of the eluent l-aminoacidoxidase, crotamine and phospholipase A were obtained.

When *Gyroxin* is injected intravenously it produces a typical neurological syndrome which consists of a cathaleptic attitude followed by whirling movements. This effect varies according to the amounts of toxin injected; it has been observed that with low dosis the animal shows lateral head inclination and tottering walk.

With higher dosis the mouse stretches its limbs, adopts a pleurostotonus or an opistotonus position falling at last into a crisis of intense and fast rolling movements of the body along its longitudinal axis which may last from 10 minutes up to 48 hours almost uninterrupted. Later if the dosis is sublethal the steady normalization of the animal begins.

This symptomatology is similar to that produced by a labyrinth lesion.

Phosphorus and phosphatases of serum and saliva. NELLY VON BENNEWITZ AND RINA MASSONE. *(Depto. Bioquímica, Esc. Química, Farmacia y Bioquímica, Univ. Concepción, Chile).*

Inorganic phosphate and phosphatase activities (alkaline and acid) were determined simultaneously in serum and saliva of four human groups, classified on account of their age and oral conditions.

The groups were:

1) Children, with healthy oral and dental conditions.
2) Adults, with healthy oral conditions.
3) Adults, with non healthy oral conditions.
4) Adults, edentates, which carry total plates.

The inorganic phosphate was determined according to the method of Fiske and Subbarrow and the phosphatase activity, to the mehod of Shinowara, Jones and Reinhard.

The serum inorganic phosphate was normal in all the examined cases. Normal values for the salivary inorganic phosphate were obtained although the phosphorus levels of saliva showed very dispersed values. The means of salivary inorganic phosphate are four or six times higher than the values of the inorganic phosphate of the serum.

The phosphatase activity, both alkaline and acid, in serum, showed normal values in every examined case.

The salivary acid phosphatase showed a more intensive activity than the alkaline in the first three groups. In the fourth, edentates, the salivary acid phosphatase did not show any activity.

Mechanism of bile rhythmic crystallization in lithiasic and non-lithiasic patients. N. BLUMENKRANTZ AND A. B. HOUSSAY. *(Cent. de Endocrinología, Min. de Asist. Soc. y Sal. Pública, Godoy Cruz 1221, Buenos Aires, Argentina).*

Crystallization of bile obtained by duodenal catheterization was studied in lithiasic and non-lithiasic subjects. Total mucoproteins were determined by the tryptophane method (Shetlar, Foster, Everett), the degree of polymerization was assessed by the toluidine blue method (Blumenkrantz) and pH measurements were also done.

The bile of lithiasic patients exhibited an "unbranched lineal type" crystallization, a lower mucoprotein content with a very low degree of polymerization and a higher pH.

In the bile of non-lithiasic patients the crystallization was either of the choral or fern-like or choral-fern like type with a higher mucoprotein content and a higher degree of polymerization, with a lower pH.

This difference showed to be of practical importance for the diagnosis of bile lithiasis.

It was demonstrated that the rhythmic crystallization of the "lineal type" corresponded to the Mg SO4 used, in the presence of the bile macromolecules in a state of very low degree of polymerization and in low concentration occurring in lithiasis.

Gall bladder bile-obtained surgically in lithiasic and non-lithiasic patients was studied and it failed to show the "lineal type" crystallization.

This work was supported by a "Fight for Sight Grant-in-Aid of the National Council to Combat Blindness, Inc", New York City.

Aislamiento y purificación del semialdehido glutámico. J. CABELLO, V. PRAJOUX Y M. PLAZA. *(Inst. Química Fisiológica Patológica, Esc. Medicina, Univ. Chile, Santiago, Chile).*

Los cultivos del mutante *Escherichia coli 55/1* acumulan semialdehido glutámico por estar bloqueada la transformación de este compuesto en prolina.

Incubando esta cepa a 37° con agitación se obtiene a las 24 horas una concentración máxima de glutámico semialdehido en equilibrio con el ácido Δ^1 pirrolin 5-carboxílico, demostrable por las reacciones coloreadas con el o-amino benzaldehido y con el p-dimetil amino benzaldehido.

Una vez centrifugadas las células bacterianas, el medio se acidifica con HCl hasta concentración 1 N; se pasa por una columna de Dowex 50 X-8. Se lava con agua y HCl 0.01 N y se eluye con HCl 0.5 N. Las fracciones que dan reacción positiva con el o-amino benzaldehido se reúnen y se evaporan al vacío sobre hidróxido de potasio.

Se obtiene un residuo de color blanco que

se reduce a prolina con borohidrido de potasio y que da las reacciones del glutámico semialdehido y su producto de ciclización, el ácido Δ^1 pirrolin 5-carboxílico.

Phosphorylation of free serine by a protein factor and ATP. G. CONTRERAS (**), ELENA MERY (*), ADELA OHLBAUM (**) AND J. THOÁ (*). *(Inst. Física y Matemáticas (*) Univ. Chile, Inst. Bacteriológico de Chile (**), Santiago, Chile).*

The phosphorylation of serine was studied in a system containing the amino acid, ATP and a protein factor obtained from Hella cells by ammonium sulphate precipitation (45 % saturation) of acetone-butanol powder. The product of the reaction was identified as phosphoserine by means of paper and c olumm chromatography and radioactive phosphorus.

The reaction has an optimal time between 20 and 40 minutes and an optimal temperature around 37° C. The reaction depends on the concentration of the protein factor and that of phosphate during the incubation.

The enzymatic nature of the protein factor is discussed.

Hidrólisis enzimática de eniaces pirofosfóricos por enzimas de la papa. A. T. DE CORI Y O. CORI. *(Cat. Bioquímica General, Fac. Química y Farmacia, Univ. Chile, Santiago, Chile).*

Se elabora un método de fraccionamiento alcohólico y otro cetónico a fin de tener separación de actividad adenosintrifosfatásica de adenosindifosfatásica.

Ambas actividades caen en la misma fracción. El patrón de fraccionamiento se repite con ambos solventes.

Se prepara adenosintrifosfato marcado en el fósforo gama con fosfoglicerilfosfato radioactivo en el fosfato del carbono 1; y una preparación de músculo libre de mioquinasa.

Con este ATP^{32} así marcado se estudia la cinética de la liberación de fósforo inorgánico del ATP.

Se discute el mecanismo de reacción de la aspirasa de papa.

(Este trabajo ha sido financiado en parte por la donación RG 5631 del Servicio de Salud Pública de los EE.UU. de Norte América.)

Papel de diversos nucleótidos en la síntesis enzimática de fosfocreatina. O. CORI. *(Cat. Bioquímica General, Fac. Química y Farmacia, Univ. Chile, Santiago, Chile).*

La síntesis de fosfocreatina a partir de ácido enol-fosfopirúvico y creatina es catalizada por un sistema enzimático soluble de músculo esquelético de rata, tanto en presencia de ADP como de DPN o de adenosin-difosforilribosa (ADP-R). Si se usa quinasa pirúvica cristalina y creatinfosfoquinasa, solamente el ADP cataliza la transfosforilación.

La preincubación del nucleótico (sin dador de fosfato) inactiva al ADP, en tanto que activa al DPN. En incubaciones hasta de 4 horas con DPN marcado con P^{32} no se puede detectar aparición de otros compuestos. Este incubado no permite transfosforilación en presencia de las mencionadas enzimas cristalinas.

La inactivación del sistema se debe a inactivación del ADP y no de la enzima, ya que el agregado de DPN reactiva al sistema.

La preincubación del nucleótido con la enzima de rata y dador de fosfato (fosfo-enolpiruvato) produce un compuesto capaz de acelerar la síntesis de fosfocreatina con enzimas cristalinas.

Se discuten estos resultados en términos de un posible mecanismo de transfosforilación no ligado al sistema ADP-ATP.

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A micromethod for the determination of bradykininogen in blood plasma. C. R. DINIZ, I. F. CARVALHO, J. RYAN AND M. ROCHA E SILVA. *(Deptos. Farmacología & Medicina, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

A micromethod for the determination of bradykininogen was developed by the assay of bradykinin liberated after the enzymatic action of trypsin upon the plasma. The main difficulty found to obtain a maximum amount

of bradykinin by the trypsin action was the interference with trypsin inhibitors and bradykininolytic enzymes present in blood. This difficulty was avoided by heating the plasma at 96 °C. This procedure according with van Arman (1955) destroys the interfering substances.

Venous blood (5 ml) is collected in a siliconed syringe moisted with a solution containing 20 units of heparin and immediately transferred to a 15 ml siliconed glass or polyethylene tube. After centrifugation 0.2 ml of plasma are transferred to 18 x 150 mm glass tube containing 1.8 ml of 0.2 % acetic acid. The mixture is heated in a boiling water bath (96° C) during 30 minutes, cooled to room temperature and then adjusted with NaOH to pH 7.30 - 7.50 and then buffered to pH 7.80 with 0.5 ml Tris buffer (0.2 M). The suspension formed is incubated at 37° C with 0.2 mg of crystalline trypsin (Worthington Labs.), during 30 minutes. At the end of this period 5 ml of boiling alcohol are added to the clear digest to 70° C during 5 minutes. The solution is then transferred to 125 ml round bottom flasks and evaporated to dryness with reduced pressure. The dried material is suspended in 4 ml of saline and the liberated bradykinin assayed biologically in a guinea pig ileum preparation by the 2 + 2 bio-assay described by Rocha e Silva (1949).

The reference standard used was a bradykinin preparation containing 3.0 U/ml (1.0 U = 0.5 μg of synthetic bradykinin, Sandoz). A unit of bradykininogen is defined as the amount of bradykininogen able to liberate 1.0 unit of bradykinin. The average concentration of bradykininogen found in 15 normal men was 21.2 units/ml (range 14.8 - 28.5 Units/ml). In other mammals the concentration was found between 4.0 units/ml (rat) and 28.9 units/ml (ox).

Metabolismo de proteínas del plasma en el bazo aislado y perfundido de rata. E. Espinosa Elorza. *(Lab. Fisiopatología, Esc. Medicina, Univ. Chile, Santiago, Chile).*

Se desarrolló un método de perfusión de bazo aislado de rata con sangre homóloga con objeto de estudiar la participación de este órgano en el metabolismo de las proteínas del plasma.

El órgano catabolizó albúmina marcada con I^{131} y desnaturalizada por calentamiento e incorporó aminoácidos marcados con C^{14} a las proteínas del plasma durante 4 — 4½ horas de perfusión. Ambos procesos se efectuaron a velocidad constante y fueron precedidos por un período de latencia de 20 — 30 minutos.

Al cabo de 4½ horas de perfusión, 1 — 3 % de la dosis trazadora se incorporó a proteínas totales del plasma y 3.5 — 5.4 % a proteínas esplénicas. Mediante fraccionamiento de las proteínas del plasma por cromatografía en dietilaminoetil celulosa, electroforesis en zona y electroforesis en papel se puso en evidencia que la radioactividad se incorporó a proteínas plasmáticas de los grupos gama, beta y alfa globulina, siendo insignificantes los valores de radioactividad incorporada a albúmina. En uno de los experimentos se calculó en forma aproximada que la producción de globulinas totales fué de 9.5 mg/24 horas, de los cuales 5.1 mg/24 hs. correspondieron a gama globulinas. Esta última cifra representa 17 % de la velocidad de recambio de gama globulinas *in vivo*.

The estimation of the bradykininogen of the plasma. J. C. Fasciolo, J. Espada and O. Carretero. *(Depto. Fisiología, Fac. Medicina, Univ. de Cuyo, Mendoza, Argentina).*

The estimation of the bradykininogen contents of the blood plasma is made difficult by the presence in it of a potent trypsin inhibitor, and by the existence of peptidases which will destroy the bradykinin formed.

Since trypsin maintains its bradykinin forming capacity on a heated sustrate, both inconveniences can be eliminated by heating the plasma in a boiling water bath, during a couple of minutes.

The method employed for the estimation of bradykininogen is as follows: 0.1 ml of blood plasma are diluted with 3.4 ml of saline solution buffered to pH 7.4 with 10 % of a N/10 solution of phosphate buffer (merthiolate for a final concentration of 1/10 000 is added as a preservative).

Tubes are heated in a boiling water bath (97° C in Mendoza), during 3 minutes. After cooling, 0.5 mg of crystalline trypsin in 0.5 ml of saline are added. The tubes are incubated

in a thermostat at 37° C during 30 minutes and afterwards put in a boiling water bath for 3 or 4 minutes, to destroy the trypsin. The bradykinin formed is estimated by its vasodilator action on a perfused dog's leg.

The plasma of the dog contains some 80-90 depressor units per ml, which corresponds to a 6-7 μg of the Boissonnas et al. synthetic bradykinin (Sandoz).

Bilateral nephrectomy, which increases markedly the angiotensinogen contents of the dog's plasma, does not produce important changes in the bradykininogen.

Ribotídio de histamina: biossíntese e caracterização. J. F. FERNÁNDEZ E O. Y. CASTELLANI. *(Depto. Histologia Embriologia, Fac. Medicina São Paulo, Univ. São Paulo, Brasil).*

A histamina, bem como o imidazol-acetato reagem com uma forma ativada de ribose, 5-fosforibosil-1-pirofosfato (PRPP) dando lugar à formação de um novo composto, o ribotídio de histamina. A reação enzimática é catalisada por extratos de mucosa de íleo de diversos animais o se processa de acôrdo com a equação:

$$\text{Histamina} + \text{PRPP} \xrightarrow{\text{PP}} \text{Histamina-Ribose-fosfato (Ribotídio)}.$$

A reação requer ATP e é inibida por aminoguanidina, Tris. O ribotídio formado é muito resistente à hidrólise ácida e mostrou conter proporções equimoleculares de histamina, ribose e fosfato. Êste último é libertado do ribotídio através da ação de 5-nucleotidase.

Physico-chemical analysis of two polysaccharides from the electric organ of Electric eel. AÍDA HASSÓN *. *(Inst. Biofísica, Universidade do Brasil, Río de Janeiro, Brasil).*

Through the study of quaternary ammonium bases interaction with macromolecules of electric organ extracts of the Electric eel *(Electrophorus electricus* (L), a fraction named FY was obtained (1). Fraction FY was precipitated by alcohol added to the concentrated supernatants of the trichloroacetic acid precipitate (STCA) of these extracts. By free electrophoresis and ultracentrifugal analysis, FY showed two major components (1) which were purified on a DEAEC-column. Their analysis by chemical methods and paper chromatography (1) indicated that they were polysaccharides. One is constituted by glucose and is a glycogen analogue. The other, with equivalent amounts of glucuronic acid and N-acetylglucosamine, is an acidic polysaccharide similar to hyaluronic acid. The neutral component is designed Sf_1 and the acid, Sf_3.

Sedimentation constant determinations of Sf_1 and Sf_3 in several media (0.05 and 0.1 M sodium acetate buffer, pH 5.6, sodium phosphate buffer at pH 7.0 and different ionic strengths, and, NaCl of different molarities) showed that Sf_1 is highly polymerized with S_{20} of 60-66 Svedberg units. Sf_3 is less polymerized than the hyaluronic acids from different sources, described by other authors, and has S_{20} of 1.2-1.5 Svedberg units.

The effect of Sf_3 concentration in the sedimentation constant was studied at 0.1 ionic strength buffer solution. The plot of concentration against sedimentation constant, showed marked deviation from linearity for concentration lower than 1 %. The same was observed by Varga et al. (2) with the hyaluronic acid of vitreous humour.

The change in ionic strength has also an effect on the sedimentation of Sf_3 reaching a minimum at zero ionic strength (deionized water).

Sf_3 in solution, like other polyelectrolytes, must have its configuration changed in the presence of its counter ions. Due to the interaction of cations and the polysaccharide negative charges, at the higher ionic strengths, the internal electrostatic repulsions will be diminished and a more compact configuration obtained. At lower ionic strengths, a stretched configuration should be expected.

The number of the available charges of Sf_3, in solution, at different ionic strengths has also been determined by potentiometric titration (3).

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Interacción molecular entre tiroxina y piridoxalfosfato y su significado fisiológico. A. HOVARTH. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

L-tiroxina, tanto *in vivo* como *in vitro*, inhibe una variedad de actividades enzimáticas que requieren al piridoxalfosfato como coenzima. Estas inhibiciones no aparecen si se le inyecta a los animales tiroxinizados piridoxina en exceso, o si se añade piridoxalfosfato *in vitro* a los homogenizados, junto o después de la tiroxina.

Se presenta evidencia cromatográfica, química y de absorción ultravioleta en el espectrofotómetro Cary de registro automático, de que la causa de las inhibiciones es una combinación azometínica, del tipo base de Schiff, entre tiroxina y piridoxalfosfato. Se comenta la importancia fisiológica de este fenómeno, en cuanto al modo de acción de la tiroxina.

Cinética de la transferencia de fosforilo del ácido 1,3 difosfoglicérico a la creatina y al adenosin difosfato. M. LAGARRIGUE Y O. CORI. *(Cát. Bioquímica General, Fac. Química y Farmacia, Univ. Chile, Santiago, Chile).*

Datos obtenidos en nuestro laboratorio, sugieren la idea de la existencia de un mecanismo de transfosforilación desde el ácido 1.3-difosfoglicérico a la creatina distinto del de Lohman y en el cual intervendría el DPN como agente transfosforilante.

Se estudia la cinética de la reacción de síntesis de fosfocreatina, utilizando una fracción soluble de músculo esquelético de rata. Al variar las concentraciones de DPN y de ATP, se observan dos grupos de resultados.

En una primera disposición experimental los resultados demuestran que el ATP sería el precursor, ya que su actividad específica es constantemente mayor a la actividad específica de la fosfocreatina.

En una segunda disposición experimental, los resultados demuestran que tanto el ATP como la fosfocreatina presentan inicialmente actividades específicas iguales, pero al cabo de 1 minuto las actividades específicas divergen y no se equilibran aún a los 16 minutos.

Se discute la importancia de estos resultados, para juzgar la existencia de uno o más comportamientos de nucleótidos.

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Aspectos quantitativos do metabolismo protéico no rato. A. A. C. LAVRAS, S. B. HENRIQUS, E. P. CAMARGO Y O. B. HENRIQUES.

Após a injeção de glicina radioativa a ratos foram isolados dos fígados dos mesmos a glicina livre, a glutationa, as proteínas microssômicas solúveis em desoxicolato (DOCS), as proteínas microssômicas insolúveis em desoxicolato (DOCI), bem como as proteínas solúveis do citoplasma das células hepáticas (PC). Por meio da técnica de Work e Campbell isolou-se a dinitrofenil-glicina proveniente da glicina hepática livre e também as dinitrofenil-glicinas obtidas por hidrólise da glutationa hepática, de DOCS, de DOCI e de PC, e determinou-se a radiatividade de tôdas as amostras.

Considerando-se válida a equação de Zilversmit-Entenman, a análise matemática dos resultados revelou que se pode aceitar a glicina livre como precursor imediato das glicinas presentes na glutationa e nas frações protêicas DOCS, DOCI e PC, ao passo que se pode excluir a glicina glutatiônica como precursora imediata da glicina contida nas mencionadas frações protêicas.

A comparação das atividades específicas das glicinas de DOCS e DOCI (ribonucleoproteínas) indica, mesmo sem o auxílio de análise matemática, que os metabolismos de ambas são independentes, não sendo os resultados obtidos compatíveis com a hipótese de ser a fração microssômica DOCI precursora da fração DOCS. Admitida, porém, a validez da equação de Zilversmit-Entenman, os resultados obtidos não são compatíveis com a hipótese de ser DOCS precursor das proteínas solúveis do citoplasma e não permiten rejeitar DOCI como precursora de PC.

Actividad del difosfopiridín nucleótido y sus productos de hidrólisis en la síntesis enzimática de fosfocreatina. F. MARCUS Y O. CORI. *(Cát. Bioquímica*

General, Fac. Química y Farmacia, Univ. Chile, Santiago, Chile).

,De los productos de hidrólisis del difosfopiridín nucleótido en diferentes medios, sólo el adenosín difosfo ribosa (ADPR) y el adenosín monofosfato (AMP) son capaces de catalizar la síntesis de fosfocreatina a partir de ácido enol fosfopirúvico en presencia de un preparado enzimático soluble de músculo esquelético de rata.

El nivel mínimo requerido en el caso del ADPR es 3.6 x 10^{-6}M y 10^{-5}M para el AMP. El ADP también cataliza esta síntesis y su nivel mínimo es de 10^{-6}M.

Si en lugar del preparado enzimático indicado anteriormente se utiliza creatínfosfoquinasa y quinasa pirúvica cristalinas, tanto el ADP, como el AMP dan lugar a síntesis de fosfocreatina. Este último resultado podría explicarse por los resultados de Molnar y Lorand (Fed. Proc. 1960, *19*, 260). En cambio el ADPR resulta inactivo con las enzimas cristalinas.

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Purificação e propriedades da fumarase de *Propionibacterium pentosaceum*. G. C. MELLO AYRES E F. J. S. LARA. *(Depto. Bioquímica, Fac. Medicina, Univ. São Paulo, Riberão Prêto, SP, Brasil).*

É descrito um método que permite uma purificação de 500 vêzes da fumarase de *P. pentosaceum*.

A reação catalisada pela enzima é fortemente influenciada por anions. Grupos -SH são essenciais para ação enzimática.

Foram efetuadas determinações de constantes de dissociação do complexo enzimasubstrato.

Effect of nonanoic acid in the fatty acids metabolism of rats fed with a diet deprived of essential fatty acids. O. MERCURI AND R. BRENNER. *(Cát. de Química Biológica; Inst. de Fisiología, Fac. de Ciencias Médicas, La Plata, Argentina).*

In a previous work we observed that the 3 cis nonanoic acid, which has a double bond in the same position that the $\triangle^6 CH_3$ of linoleic and arachidonic, produced a remarkable increase in the arachidonic's content of rats deprived of essential fatty acids. In this experiment we tested the respective saturated acid, nonanoic acid and heptanoic acid. We studied by gas-liquid chromatography, the lipids of heart, liver and fat depots of controls, deprived rats, deprived rats plus nonanoic and deprived rats plus linoleic acid. We observed that, although the diet influences the composition of the epididimal fat pad, the nonanoic and the heptanoic did not increase the content of odd fatty acids and therefore they must be quickly metabolized. The linoleic acid is stored in low proportion and the poliethylenic acids are not practically accumulated. In the liver the lack of essential acids is shown by a decrease of linoleic and arachidonic acids and the appearance of eicosatrienoic. The nonanoic and heptanoic acids raise somewhat the proportion of linoleic and arachidonic and also increase the eicosatrienoic acids. In the heart, the absence of essential fatty acids is more evident and very similar to the liver's reaction, although here the effect of odd acids seems to exaggerate the deficiency. We also observed a great increase of oleic acid. The addition of linoleic acid corrects the effects of the deficiency increasing the proportion of linoleic and arachidonic acids. We also observed an interesting increase of an acid of 22 carbons and 5 double bonds, in liver and heart. Histological studies were done at the same time in testicles, aorta and liver. The heptanoic and nonanoic acids seem to be mainly degraded in the conditions of the experiment and not used to build longer fatty acids.

Activity of different yeast protein fractions in the phosphorlylation of serine. E. MERY AND J. TOHÁ C. *(Inst. Física y Matemáticas, Universidad de Chile, Santiago, Chile).*

The formation of phosphoserine is described in a system containing serine, ATP and a baker's yeast extract or the protein fraction

precipitated with 45 % saturation ammonium sulphate.

The product of the reaction is identified as phosphoserine 1 - C14 by paper chromatography. After dialysis, the protein fraction lost the activity.

Quantitative changes in liver phosphorylase determined by modifications in the diet. H. NIEMEYER, NORMA PÉREZ, J. RADOJKOVIC AND T. URETA. *(Inst. Química Fisiológica y Patológica, Univ. de Chile, Borgoño 1470, Santiago, Chile).*

Liver phosphorylase activity depends on the caloric intake of the animal. The enzyme content was 729 + 9.9 Units/100 gm body weight in rats receiving about 35 Cal/100 gm body weight in the form of equilibrated diet; and was only 537 + 4.6 U in the animals with an intake of about 24 Cal.

Rats fed for 6 or 10 days an exclusive carbohydrate diet exhibited values of phosphorylase (495 ± 25 and 552 ± 39 U respectively) similar to those of animals fed isocaloric amounts of equilibrated diet, in spite of the protein depletion. Groups of rats fed fat-free carbohydrate diets with variable proportions of casein showed about the same figures.

On the contrary, the enzyme activity decreased markedly in rats kept on a carbohydrate-free high-fat diet (330 ± 18 and 405 ± 9 U in animals receiving about 24 and 31 Cal respectively). Even after only 1 day on this diet the diminution may be observed.

The rate of increase in the phosphorylase content of the liver after a decrease provoked by a 48 hours fasting was the same in animals refed an equilibrated diet or carbohydrates alone. In the latter condition the liver proteins remained at the same low levels as during fasting.

Data will be discussed considering the possibility of an adaptative response of the liver cells to the nature of the diet.

Role of inactivation in the decrease of liver phosphorylase by several dietary conditions. H. NIEMEYER AND J. RADOJKOVIC. *(Inst. Química Fisiológica y Patológica, Univ. de Chile, Borgoño 1470, Santiago, Chile).*

It has been demonstrated that the phosphorylase activity of the liver decreases in rats fasted for 24 hours or more and in those fed a high-fat diet. The possibility was considered that the diminished activity was a reflection of a lower proportion of the active form with respect to the total phosphorylase protein. As it is known that epinephrine is able to activate phosphorylase under certain circumstances, it was tried either in the whole animal or in liver slices. The results showed that the drug was uneffective in the rat, although it was active in other mammals.

Experiments performed with liver homogenates showed that phosphorylase was inactivated at a higher rate in animals fed an equilibrated or a high-carbohydrate diet than in those fed a high-fat carbohydrate-free diet or fasted for 48 hours. The addition of ATP, Mg^{++}, KF and caffeine resulted in activation of the enzyme when it had been inactivated previously *in vitro*. However, when fresh homogenates prepared in KF to avoid inactivation were subjected to the same procedure, it was not observed any increase in phosphorylase activity either in rats fed equilibrated or high-fat carbohydrate-free diets or in fasted animals. These results are interpreted as indicating that liver phosphorylase in the rat is maximally activated under different dietary conditions.

Inativação da angiotensina por foto-oxidação. A. C. M. PAIVA E T. B. PAIVA. *(Lab. Farmacologia e Bioquímica, Esc. Paulista Medicina, São Paulo, Brasil).*

Entre os resíduos de amino-ácido presentes na angiotensina II, figura um histidil na posição 6 da cadeia peptídica, cuja importância para as atividades biológicas da angiotensina não tinha sido ainda estudada. Este problema foi agora investigado através de uma tentativa de foto-oxidação de Asparagil 1, Valil 5 angiotensina II sintética. Verificamos que soluções 5 x 10-4 M da angiotensina em tampão fosfato 0,2M (pH 8) em presença de azul de metileno 0,005%, quando irradiadas com luz visível perdem totalmente sua atividade sôbre

a pressão arterial de rato, sôbre o íleo isolado de cobaia e sôbre o útero isolado de rata. Paralelamente à perda dessas atividades há consumo de oxigênio até o máximo de 1 mol por mol de angiotensina. Determinações de tirosina e histidina em hidrolisados da angiotensina foto-oxidada, mostraram que o seu resíduo tirosil não é afetado pela foto-oxidação, enquanto que o resíduo histidil é totalmente destruído, e sua destruição é paralela à perda de atividade. Em ausência de luz, a reação não se processa. Concluimos que a presença do anel imidozólico da histidina é essencial para as atividades biológicas da angiotensina.

Estudios sobre el antibiótico bacilomicina. A. C. PALADINI, M. BURACHIK Y N. C. LEARDINI. *(Inst. Fisiología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

De los caldos de fermentación de microorganismo *B. subtilis DINR 49-4* se obtuvo un preparado impuro del antibiótico fungistático Bacilomicina A. y se realizó el estudio de su composición por distribución en contracorriente. Este método permitió demostrar la existencia mínima de tres componentes activos, muy similares entre sí, en cuya composición entran los aminoácidos aspártico, prolina, tirosina, glutámico y serina.

Partial purification and characterization of a plasma kinin liberator from horse urine. J. L. PRADO AND ELINE S. PRADO. *(Labor. Farmacología e Bioquíca, Esc. Paulista Medicina, São Paulo, Brasil).*

Normal horse urine contains a plasma kinin liberating agent with the following properties: it is a protein precipitating at pH 7.0, between 0.40 to 0.70 ammonium sulphate saturation; it may be precipitated both by ethyl alcohol and acetone and liberates rapidly a plasma kinin from horse blood plasma and plasma of a few other species. The kinin liberation is inhibited by DFP but is not inhibited either by ovomucoid or by soy bean trypsin inhibitor. Crude preparations of this kinin liberating factor contain a kininolytic agent (Kininase)which inactivates the kinin produced from plasma; this kininase may be destroyed by a few minutes heating, at neutral pH, in a boiling water bath, leaving the liberating activity slightly diminished; it is inhibited by cysteine. The kinin liberating activity is assayed by mixing known amounts with an excess of kininase-free horse plasma in the muscle chamber where a piece of guinea-pig ileum is suspended in Tyrode solution at 37° C; the amount of liberated kinin is compared with that produced by a preparation taken as standard. It is also possible to incubate the preparations and plasma, both without kininase, for a few minutes, and assay the kinin formed following interruption of the reaction. Finally, an approximate assay may be obtained from the hypotensive action of the preparations in the dog.

The liberating agent exhibits esterolytic activity both on TAME and BAEE, which serves as the basis for a biochemical assay. A parallelism was found between the esterolytic activities of different preparations and their potencies obtained from the biologic assays.

From experiments on the amount of liberated kinin, the optimum pH of the liberating agent is in the range pH 8-9.

We are not dealing with urokinase, a plasminogen activator found in urine. Judging from all the properties above described, we believe that the liberating agent is urinary kallikrein and the kinin formed is kallidin.

In a previous work (2) we had purified 80 times horse urinary kallikrein, using ammonium sulphate fractionation of urine followed by purification on DEAE-cellulose columns. We have introduced now a fractionation of the preparation with acetone, at room temperature, previous to the DEAE-cellulose column fractionation; we have thus obtained a preparation with a final specific activity of 300-400, if one considers the specific activity of the ammonium sulphate precipitate as unity.

The esterolytic activity of the purest preparation on BAEE is comparable to that of crystalline trypsin, a degree of purification not yet obtained for urinary kallikrein. The preparation exhibited no proteolytic activity on casein.

(1) Work supported by grant RF nº 58217 from the Rockefeller Foundation.

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Purification and chemical analysis of antigenic fractions from *Ascaris lumbricoides*. J. PUDLES, W. DIAS DA SILVA AND W. T. BERALDO. *(Deptos. Bioquímica e Fisiología, Fac. Medicina, UMG, Belo Horizonte, Brasil).*

Gazzinelli *et al.* (1961), using a column of hidroxyapatite, isolated an active fraction from *Ascaris lumbricoides* which caused an anaphylactic-like reaction on the guinea pig. The chemical analysis of this fraction (SB_4) showed a high content of reducing sugars. In the present paper further purification of the active *Ascaris* fraction was tried. Using phenol and water extractions employed for separation of protein from polysaccharide, according to Westphal *et al.* (1952) we were able to isolate two fractions. One of them was soluble in phenol, very rich in protein (SF_1) and the other one soluble in water containing polysaccharides (SFH_2O). The SF_1 fraction was run through a column of Amberlite IRC50 x E64 (200-400 mesh) and after elution 3 protein peaks were obtained. The activity was found only in the first peak. The SFH_2O fraction presented the biological and chemical properties similar to that found in the SB_4 fraction described by Gazzinelli *et al.*

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Contribución al estudio del catabolismo de las catecolaminas. J. C. RAMASSO Y A. E. DOMÍNGUEZ. *(Inst. Fisiología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Se perfunden tejidos de sapos *Bufo arenarum Hensel* con inhibidores de distintas enzimas, con diversas técnicas, a distintas dosis, en experiencias agudas y crónicas.

Se estudia el catabolismo de la adrenalina en los tejidos, se discuten los mecanismos enzimáticos responsables y además las variaciones en la glucogenolisis espontánea y adrenalínica.

Acción del DNP sobre la oxidación de sustratos endógenos por *Saccharomyces cerevisiae*. E. H. RAMOS. *(Inst. Química Biológica, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

El 2-4 dinitrofenol (DNP) 10^{-4} M inhibe la oxidación del ácido acético y la operación del ciclo del ácido cítrico en la levadura *Saccharomyces cerevisiae* cuando el medio es suficientemente ácido (pH 4.5). En oposición a ese efecto, la adición del DNP a levadura preincubada con acetato-1-C^{14} y acetato-2-C^{14} provoca a) aumento del consumo de oxígeno correspondiente a la oxidación de sustratos endógenos y b) aumento de la producción de CO_2 radiactivo sin modificar la actividad específica del mismo. El agregado de DNP no modifica el contenido de aminoácidos libres en las células como lo demuestra su valoración pero disminuye el contenido de hidratos de carbono que se pueden extraer con TCA 5 % en frío y a 100°C. En las células preincubadas con acetato-C^{14} el DNP modifica la distribución de C^{14} en los reservorios metabólicos, pues disminuye la rotulación de los ácidos glutámico y alanina y aumenta la de los ácidos tricarboxílicos y el aspártico.

Vias alternativas para oxidação do DPNH no fígado. I. RAW. *(Lab. Enzimologia, Fac. Medicina, Univ. São Paulo, São Paulo, Brasil).*

Várias flavoproteinas foram isoladas de fígado, que oxidam DPNH. Suas propriedades não corresponden às previstas para o sistema de oxidação fosforilativa presente no condrioma.

Foi isolada uma enzima que oxida DPNH reduzindo quinonas (menadiona, naftoquinona, coenzima Q). Essa enzima bastante instável é inibida por dicumarol, adeninonucleotidos, quinacrina e p-clomercuribenzoato. Suas propriedades são distintas da DPNH-citocromo b5 reductase e da DPNH-TPNH diaforase. A relação entre essa enzima e a flavoproteina da cadeia fosforilativa será discutida.

Identification of enzyme, in ophidian venoms, responsible for ADP dephosphorylation. S. SCHENBERG AND F. A. PE-

REIRA LIMA. *(Secc. Fisiología, Instituto Butantan, São Paulo. Brasil).*

The enzymic process by means of which ophidian venoms dephosphorylate ADP is not yet well established. E. A. Zeller (1) states that ATP and ADP are both actuated by ophio-ATPase. M. Johnson *et al.* (2) suggest the existence of an ADPase in *Naia naia* venom.

Brazilian snake venoms have small ADPase activity, however, their ATPase activity is relatively strong. The study of both enzymes in these venoms presents some difficulties due to their similar characteristics and low ADPase activity.

Other investigations showed that single snake venoms may not contain one or more of the components found in their species venom pool (3, 4). Based on these observations and trying to simplify the analytic part, individual venoms from several species were investigated with the finality to find venoms which would only present ADPase activity being, however, deprived of ophio-ATPase; this was attained with single venoms of *Bothrops cotiara* species.

This finding, by itself, was sufficient to confirm the existence of two distinct enzymic processes responsible for ATP and ADP dephosphorylation. With these venoms it was also observed that the ADPase activity presents some characteristics, Co^{++}, constituting its best activator, increasing ADP dephosphorylation in about 10 times, this ion activates to a lesser degree ophio-ATPase from these venoms.

The low ADPase activity of these venoms turned uninteresting their study. Another more active venom was then searched and this of *Bitis gabonica* showed to be well fitted for this investigation as it strongly dephosphorylates AMP (5-nucleotidase), ATP (ophio-ATPase) and ADP (ADPase activity), allowing a better comparative study of these phosphatases.

Characteristics of "Bitis gabonica" activity. The substrates and their degradation products were separated by paper chromatography and estimated by ultra-violet light in a Beckman spectrophotometer. In diluted solutions, at room temperature, this venom looses, in the first hour, about 70 % of its ADPase activity. The determinations were made with solutions containing 10-30 μg/ml of venom. Incubations were made with glycine buffer pH 8.8, during 15 minutes, in a waterbath at 37° C.

Activation. Co^{++}, Mg^{++}, Mn^{++} and Ni^{++} are activators. Co^{++} in 4 mM concentration is its outstanding activator, increasing about 27 times (2700 %) its ADPase activity.

Inactivation. Zn^{++}, Cd^{++}, cysteine and glutathione are inactivators. Cysteine is the best inactivator, cystine and oxidized glutathione are both inactive, this suggests the possibility of SH groups to be involved in the enzymic process.

Differences between ADPase activity and ATPase from "Bitis gabonica" venom. 1) Considering venom weight, ADPase activity is much stronger than ATPase; 2) Co^{++} activates much more the ADPase activity than ATPase; 3) Mg^{++} activates less ADPase activity than Co^{++}, both these ions activate equivalently ATPase; 4) Zn^{++} inhibits ADPase activity and is one of the best ATPase activators; 5) $\alpha\,\alpha'$ dipyridyl is indifferent for ADPase activity but strongly inhibits ATPase; 6) ADPase activity is found in venoms deprived of ophio-ATPase.

Thiamine pyro and orthophosphates are not substrates for ADPase activity, this seems to show some specificity for ADP, meanwhile, as this enzyme characteristics are not well established, it was provisorily termed by its activity (ADPase activity); further investigations will confirm if it can really be considered as a true ADPase.

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Propiedades de algunas aldehido-deshidrogenasas. M. N. SCHWARCZ Y A. O. M. STOPPANI. *(Inst. Química Biológica, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

La DPN aldehido deshidrogenasa de levadura es activada por complejantes de metales (cisteina, 1,10-fenantrolina, 8-hidroxiquinolina dietilditiocarbamato, EDTA, BAL, etc.) con DPN, DeamDPN, APyDPN y APydeamDPN como aceptadores de hidrógeno. Los complejantes compiten respecto a un sitio común de acción. La 1,10-fenantrolina activa la enzima promoviendo una asociación más estrecha del DPN con la enzima. En concentraciones altas, los complejantes inhiben la reacción enzimá-

tica por competencia con el DPN. Por el contrario, no modifican la constante de disociación aparente del compuesto enzima-acetaldehido. En presencia de complejantes aumenta la reactividad de los tioles enzimáticos, que intervienen en la unión con la coenzima. Con la TPN-aldehido deshidrogenasa de levadura, hay una competencia similar entre los complejantes y el TPN, pero sin acción activadora de los complejantes.

A desidrogenase láctica de *Propionibacterium pentosaceum*. A. SERZEDELLO, R. MOLINARI E F. J. S. LARA. *(Depto. Bioquimica, Fac. Medicina, Univ. São Paulo, Ribeirão Prêto, SP, Brasil).*

A desidrogenase láctica de *P. pentosaceum* foi purificada extensivamente. As preparações obtidas reagem diretamente com sulfato de metilfenazônio e azul de metileno e indiretamente com outros receptores de eléctrons.

Vitamina K e "Kochsaft" de preparações relativamente impuras de enzima, podem mediar o transporte de eléctrons entre a desidrogenase primária e 2,6-diclorofenolindofenol e 1,2-naftoquinona-4-sulfonato.

Em condições anaeróbicas, a enzima catalisa a redução de Vitamina K_1 por lactato, havendo correspondência estequiométrica entre o piruvato, formado e a vitamina adicionada à reação.

Mecanismo de acción de algunas hormonas esteroides sobre los sistemas DPNH —oxidasa y succinoxidasa de tejidos animales. A. O. M. STOPPANI, J. A. BRIGNONE, C. C. BRIGNONE Y B. N. BADANO. *(Inst. Química Biológica, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

El estilbestrol, la estrona, progesterona, desoxicorticosterona y testosterona en concentración del orden μM inhiben fuertemente la DPNH-oxidasa de miocardio y en menor grado la succinoxidasa. La cortisona es mucho menos activa y la prednisona, prednisolona y triamcinolona carecen de acción. Preparaciones de DPNH-oxidasa de cerebro y músculo esquelético son inhibidas en igual forma poi los esteroides mencionados en primer término mientras que las preparaciones de testículo, hipófisis, hígado, riñón, tiroides, suprarrenal y bazo son mucho menos sensibles o no son afectadas. Sin embargo, los esteroides inhiben la DPNH-oxidasa de miocardio y en menor grado la succinoxidasa de hígado y riñón, cuando la actividad se mide en condiciones que aseguran la estructura de las partículas. Con DPNH-oxidasa de miocardio, la preincubación con estilbestrol, estrona y progesterona determina una disminución de la actividad enzimática, a pesar de la eliminación de la hormona. Con hormonas rotuladas con C^{14} se comprueba la fijación de la hormona al sistema enzimático. Por inhibición directa o con preincubación no se demuestra acción de los esteroides sobre la DPNH-deshidrogenasa DPNH-citocromo *c* reductasa, succino deshidrogenasa o citocromo oxidasa lo que implica una acción selectiva sobre el mecanismo de acoplamiento de las flavoproteinas al sistema de los citocromos. La remoción total con acetona del C^{14} fijado en preparaciones preincubadas con hormonas radiactivas concuerda con esa hipótesis pues el acoplamiento mencionado se efectúa mediante lipoproteinas.

CIRCULACIÓN Y RESPIRACIÓN
CIRCULATION AND RESPIRATION

Effects of several central nervous system stimulants and depressant drugs upon the heart rate in rats. J. ALDUNATE, H. HERRERA AND A. SALINAS. *(Inst. Farmacologia, Univ. Chile, Santiago, Chile).*

Previous work has shown that the continuous i.v. injection of several C.N.S. depressant and stimulant drugs produced a gradual slowing of the heart rate under urethane anesthesia.

The present paper deals with the influence of vagotomy and atropin on this bradycardia. Four groups of rats were used: the first one with section of both vagi nerves in the neck; a second group with sham operation

and intact vagi nerves; a third group similar to the second one, but receiving 1 mg/kg of atropine i.p., and a control group similar to the third one, but receiving distilled water i.v. instead of atropine.

The following drugs were tested. nikethamide, strychnine, picrotoxin, morphine, methadone, thiopental, imipramine, iproniazid, chlorpromazine, mephenesine, meprobamate and ethanol. During the administration of the drugs the EKG was continuously recorded.

All the tested drugs produced a decrease in the heart rate and this effect was not prevented by section of vagi nerves or by atropine. When nikethamide, methadone, morphine or picrotoxin were given, the slowing of the heart was less marked after atropine or bilateral vagotomy. No change in the heart rate was observed in the control group.

In order to know whether this bradycardia was the consequence of a direct action of these drugs on the atrial sinus, their effect on the frequency of the isolated rat auricle with and without previous addition of atropine (0.002 mg/ml) was studied. The rate of this preparation was recorded by an electrogram. All the drugs tested induced a decrease in the atrial rate, even in the presence of concentrations of atropine high enough to inhibit the effect of acetylcholine.

Modificación de la acción cardioestimulante de la acetilcolina por inhibidores de las amino oxidasas. F. ALVARADO y S. MIDDLETON. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

Se analizó la acción inotropa positiva y la liberación concomitante de substancia adrenalinosímil, inducidas por la inyección intracoronaria de acetilcolina en corazones aislados, atropinizados, perfundidos con solución Tyrode, de gatos inyectados previamente (2 horas antes) con nialamida (Pfizer) endovenosa (10 mg/kg peso).

La inyección intracoronaria de 20 microgramos de acetilcolina, indujo un aumento promedio de amplitud de contracciones de 162 % en 10 corazones "nialaminizados" y de 83.1 % en 10 corazones "no nialaminizados" (t 5.81, $p < .001$). La liberación concomitante de substancia adrenalinosímil, estimada por la disminución del tono del ciego rectal de pollo inducida por los perfusados cardíacos, equivalió a una concentración promedio de adrenalina de 2.4×10^{-9} en los "nialaminizados" y de 1×10^{-9} en los "no nialaminizados" (t 4.8, $p < .001$). El efecto sobre el tono cecal fué abolido por nicotinización y por ergotaminización del intestino. 50 microgramos de acetilcolina tuvieron, también, efectos significativamente mayores en los corazones "nialaminizados".

Estos resultados podrían interpretarse como debidos a que la nialamida, inhibiendo las monoaminoxidasas, aumentaría el contenido catecolamínico cardíaco.

Los efectos inotropos positivos de dosis submáximas de adrenalina y noradrenalina, no mostraron diferencias significativas entre los corazones "nialaminizados" y los "no nialaminizados".

Efecto de la reserpina y de la adrenalectomía sobre la acción cardioestimulante de la acetilcolina. F. ALVARADO, S. MIDDLETON y J. P. BECA. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

Es un hecho establecido que el efecto inótropo positivo inducido por la acetilcolina en el corazón atropinizado de mamífero, es tanto mayor cuanto mayor es la liberación concomitante de substancia adrenalinosímil. Se sabe que el contenido catecólico cardíaco disminuye marcadamente por acción de la reserpina y moderadamente por extirpación de la médula suprarrenal. Pareció de interés analizar la influencia de la reserpina y de la adrenalectomía sobre los efectos de la acetilcolina en el corazón atropinizado.

Se trabajó en el corazón aislado, atropinizado, perfundido con solución Tyrode, de gatos adultos de ambos sexos. La liberación de substancia adrenalinosímil, se estimó por la disminución del tono del ciego rectal de pollo producida por los perfusados cardíacos.

En 10 corazones de gatos inyectados 24 horas antes con reserpina endovenosa (1 mg/kg peso) la inyección intracoronaria de acetilcolina en dosis de 10 a 100 microgramos, no ejerció en ningún caso su efecto estimulante habitual. Tampoco se detectó liberación de substancia adrenalinosímil.

En 7 corazones de gatos adrenalectomizados

12-14 días antes, el aumento promedio de la amplitud de las contracciones producida por 50 microgramos de acetilcolina fué de 58 %; en 13 corazones control fué de 85 % (t 4.61, $p < .001$).

La liberación de substancia adrenalinosímil equivalió a una concentración promedio de adrenalina de 3.1 x 10^{-10} en los adrenalectomizados y de 1 x 10^{-9} en los controles (t 3.7, $p < .01$).

Estudio sobre la capacidad de crecimiento del riñón con clip en la arteria renal. M. CEREIJIDO, R. FUKMAN Y E. MARTÍNEZ-PICABEA. *(Inst. Fisiología. Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Cuando se estrecha la luz de una arteria renal, el riñón afectado disminuye de tamaño. El riñón contralateral se hipertrofia. Se suele pensar que el estrechamiento de la luz arterial impide el crecimiento renal.

En el presente trabajo se toman 4 lotes de ratas blancas: I control; II nefrectomía unilateral; III clip de plata en una arteria renal y IV clip en una arterial renal y nefrectomía del riñón opuesto.

En el lote III se observó que al poner un clip en una arteria renal este riñón se atrofia y el opuesto se hipertrofia ($p < 0.001$); la masa renal total no tiene una diferencia significativa con la de los animales normales.

En el lote IV el riñón con clip se hipertrofió por encima de lo normal ($p < 0.001$); hecho interesante que demuestra que la capacidad de crecer del riñón con clip no está abolida.

Sin embargo la hipertrofia compensadora es mayor en el lote II que en el IV ($p < 0.02$).

Se agregarán a la comunicación otros datos aún en estudio y se discutirán otras posibilidades.

Cerebral vaso-dilatory effect of bradykinin in the dog. A. P. CORRADO * AND A. CARPI. *(Ist. Superiore di Sanità, Roma, Italia).*

Among the biological effects induced by bradykinin, those resulting from its action on the cardiovascular system are of particular importance (1). Recent work (2-5), which has shown the presence of a bradykinin-liberating enzyme in the perfusion fluid of certain organs, like the cerebral ventricular system of cats, suggests an important rôle for the polypeptide in the regulation of blood flow in certain areas.

The results obtained on the ventricular system, led us to investigate the effects of bradykinin on the cerebral circulation.

The reactivity of the cerebral vessels was studied in chloralosed (100 mg/kg i.v.) dogs from which simultaneous records, by means of a 6-channel Grass Polygraph, were taken: intracraneal venous pressure, arterial blood pressure, nasal plethysmogram and respiration. To measure the cerebral venous pressure, a polyethylene catheter was introduced in the cranial direction into the peripheral stump of the external jugular vein (6).

In some preparations the venous outflow of the superior sagittal sinus, the cardiac rhythm and the venous systemic pressure were also recorded.

When injected by the intravenous route bradykinin, in doses varying between 3-10 units/kg (natural product) and between 0.50-3 μg/kg (purified or synthetic products), evoked marked cerebral vaso-dilatation indicated by the pronounced increases in cerebral venous outflow and intracranial venous pressure.

Discrete respiratory stimulation and fleeting tachycardia, were also observed; however, no effect was noted on systemic venous pressure and on the nasal plethysmogram. This illustrates the slight sensivity of the vessels of the nasal mucosa to doses of bradykinin which are capable of inducing marked effects on the cerebral vessels.

All the observed effects were of a transitory nature (duration 30-60 seconds) and there was no observable tachyphylaxis following repeated administration of the polypeptide.

The vascular effects of bradykinin are not inhibited by doses of Neo-antergan or atropine which block the cerebral vasodilatation induced by histamine and acetylcholine respectively. On the other hand, it could be shown that histamine will also induce a marked dilatation of the vessels of the nasal mucosa; this phenomenon was not observed with bradykinin.

This shows the existence of a special sensitivity of the cerebral vessels to vasodilatation by bradykinin and indicates the possible important rôle of this polypetide in the regulation of the cerebral circulation.

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Efecto de la imipramina en la respuesta presora a la estimulación vagal aferente en perros reserpinizados. J. COUSSIO, A. J. KAUMANN Y J. A. IZQUIERDO. *(Cát. Farmacología Experimental, Fac. Farmacia Bioquímica, Buenos Aires, Argentina).*

En el perro, la imipramina contrarresta la hipopresión de la reserpina, antagonizando la respuesta hipopresora refleja de la estimulación vagal aferente con hiperpresión si el alcaloide se ha inyectado 1 hora antes. Este último efecto no se observa si la reserpina se ha inyectado 48 horas antes. Los efectos presores de la norepinefrina exógena son siempre más acentuados.

Se sugieren efectos periféricos y centrales de la imipramina.

Vasoactive polypeptide derived from human plasma. H. B. CROXATTO. *(Labor. Fisiología, Escuela de Medicina, Univ. Católica de Chile, Santiago, Chile).*

The incubation of human plasma under the previously described conditions (Croxatto et al., 1959) liberates a polypeptide possessing the characteristics of anephrotensin, obtained from rat serum, on account of its properties regarding proteolytic enzymes and its pharmacological effects. The blood serum has an enzyme which is irreversibly destroyed by acid incubation. This enzyme swiftly inactivates anephrotensin. The previous inactivation of this enzyme determines that the incubation of this serum at a slightly alkaline or neutral pH (7.4) gives rise to the accumulation of a polypeptide displaying features similar to those of anephrotensin, obtained in the usual way. This substance is studied comparativelv with angiotensin II (valyl 5) and synthetic bradykinin.

CROXATTO, H., PEREDA, T., and MELLADO, R.: Nature (Lond), 1959, *184*, 1496.

Effects of aldosterone and oxytocin upon the intra-extra cellular sodium gradient in human blood "in vitro". R. CROXATTO REZZIO AND A. FORADORI. *(Lab. Bioquímica, Escuela de Medicina, Univ. Católica de Chile, Santiago, Chile).*

The intravenous injection of 25 g of NaCl in normotensives induced a fall in the Na_i/Na_o gradient whereas a rise was obtained in hypertensive and primary hyperaldosteronism patients. The same effect obtained in normotensive subjects is reproduced in essential hypertensive patients if the blood pressure is decreased previously with hypotensive drugs. In a subject surgically cured of his hyperaldosteronism the same dose of NaCl, induced a fall in the Na_i/Na_o gradient as in normals.

This finding, further promoted the study "in vitro" of aldosterone and oxytocin on the Na_i/Na_o gradient in human heparinized blood. The results indicated that aldosterone (d-1 aldosterone Ciba) in a concentration of 0.05 μg/ per ml resulted in a rise in the gradient when this gradient was originally low and in a fall when it was high. Between these two opposite effects, there exists a very narrow threshold in this gradient where no response to aldosterone was observed. On substituting plasma by Tyrode solution the described effects are intensified, mainly the outward movement of the sodium of the erythrocytes towards the plasma. Oxytocin (*Syntocinon Sandoz*) at the concentration of 2 mU per ml of blood, either does not result in important changes of the sodium in the erythrocyte/plasma system or it incorporates, in contrast to aldosterone, the sodium into the erythrocyte independently from the previous gradient. This

effect favourable to the incorporation of sodium, is intensified when plasma is substituted by Tyrode solution.

Since both hormones are added to the blood simultaneously, their antagonistic trends bear on the movement of the sodium. On the other hand, oxytocin has shown to have "in vitro" a coadjuvant activity in the homeostatic regulating mechanisms of the erythrocytes/plasma system regarding basic-acid changes artificially forced.

The great influence that these hormones have shown to possess regarding the modification on the sodium gradient in the erythrocyte/plasma system "in vitro", allows us to assume that the variation of that gradient, comparatively observed between normotensive and hypertensive animals, could be partially conditioned to a rupture of the equilibrium between those humoral factors.

Volumen sanguíneo pulmonar en perros mediante el empleo de curvas de dilución en la arteria pulmonar y en la aurícula izquierda. J. D. FERMOSO, E. BALLINA, A. BARRIOS Y P. ARAMENDIA. *(Centro Investigaciones Cardiológicas, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

En 9 perros se efectuaron curvas de dilución con azul de Evans desde la arteria pulmonar por un catéter Cournand nº 8 y desde la aurícula izquierda por una aguja de Ross.

Se determinaron volumen minuto y volumen sanguíneo pulmonar. El volumen sanguíneo pulmonar se obtuvo por la diferencia entre los volúmenes de dilución de ambas curvas. Se midió la volemia a los 10 seg de realizada la primera curva de dilución.

Se analizan los resultados obtenidos y la correlación entre volumen sanguíneo pulmonar y volumen minuto.

Effects of adrenergic and adrenergic-blocking drugs upon the EEG changes induced by bleeding in rabbits. L. GOLDSTEIN AND C. MUÑOZ. *(Depart. of Pharmacology, Emory University, Atlanta, Ga., USA).*

The effect of dl-amphetamine as well as of dichloroisoproterenol (DCI), phenoxybenzamine, phentolamine and hexamethonium on the EEG changes induced by the removal of 1 ml of blood per 100 gm of body weight and the subsequent injection of equivalent amount of Dextran solution were quantitatively studied in curarized rabbits. The general procedure was the same as described in another communication reported at this meeting (see Muñoz, and Goldstein).

Previous injection of dl-amphetamine HCl (0.5 mg/kg, i.v.) did not change significantly the hypotension induced by bleeding, but it prevented the coincident increase of electrogenesis. Similar results were obtained after DCI (4 mg/kg i.v.), a beta-receptor blocking drug.

In animals pre-injected with phenoxybenzamine (2 mg/kg, i.v.) the hypotension observed after bleeding was more pronounced, but the increase of electrogenesis was of the same order as the control group. In contradistinction, the group receiving phentolamine, another alpha-receptor blocking drug, exhibited a persistent and significant decrease in electrogenesis after bleeding, although the hypotension was much more pronounced than in the control group.

Hexamethonium (0.1 to 1.0 mg/kg, i.v) markedly enhanced the hypotension and the increase of electrogenesis after bleeding as compared with the control animals. Larger doses of this drug (2-3 mg/kg) induced by themselves an important increase on the EEG voltage output which masked the electrogenesis changes observed after bleeding.

Ascorbic acid and hemorrhagic shock in dogs. II. Changes in adrenal cortex and total gland. O. A. GÓMEZ AND J. A. SANTOMÉ. *(Labor. Patología y Cirugía Exerimental, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

The ascorbic acid content of adrenal cortex and total gland was studied in experimental hypotensive hemorrhage in the dog.

A highly significant decrease below normal levels was found both in total adrenal and adrenal cortex after the experimental hemorrhage.

Reinfusion of blood after 2 h 15 min of controlled hemorrhage not only failed to restore the ascorbic acid to normal levels, but proved to be unable to prevent its dropping to lower levels.

Estudios farmacológicos con pirogalol. I. IZQUIERDO, J. A. IZQUIERDO, A. J. KAUMANN Y J. D. COUSSIO. *(Cát. Farmacología Experimental, Fac. Farmacia y Bioquímica, Buenos Aires, Argentina).*

1) El pirogalol provoca hiperpresión e inhibición intestinal fugaces semejantes a la de la epinefrina. A veces, produce leve hiperpresión sostenida de 15 a 30 minutos.

2) Potencia las acciones presoras de la epinefrina, norepinefrina y de la estimulación vagal aferente, las dos últimas en forma inconstante. En la mayoría de los casos, prolonga la taquicardia producida por epinefrina y norepinefrina, y la bradicardia por estimulación vagal aferente. La depresión y taquicardia de la isopropilnorepinefrina son potenciadas en forma constante e intensa. No modifica la hiperpresión por oclusión carotídea.

3) Secundariamente a la inhibición inicial, eleva el tono y motilidad duodenal durante 15 a 35 minutos. Este efecto sería colinérgico y periférico.

Physiology of Mollusca. I-Action of acetylcholine on the heart of *Strophocheilos oblongus*. C. P. JAEGER. *(Inst. Fisiología Experimental, Fac. Medicina, Pôrto Alegre, R. G. S., Brasil).*

A technique for the perfusion of the isolated ventricle of the snail *Strophocheilos oblongus* is presented. Acetylcholine has an excitatory action on this heart. An antagonism between acetylcholine-atropine was found for this heart. The cardio-accelerator present in *Strophocheilos* heart tissue extract is not acetylcholine.

The effect of insulin-glucose administration on the serum potassium and blood pressure responses to epinephrine. E. M. KRIEGER. *(Depto. Fisiología, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

It has been shown (1) that the administration of insulin-glucose produces a simultaneous reduction on serum potassium and a decrease in the blood pressure responses to pressor substances. The small pressure increases with low serum K were accompanied by a smaller response in peripheral resistance, the heart rate was greatly slowed and the flow greatly reduced in response to pressor stimuli. The tendency for the normalization of the blood pressure response by the slow infusion of KCl was accompanied by only a small recuperation of the serum potassium levels. The evolution of serum potassium and the blood pressure changes to epinephrine were observed in seven dogs anesthetized by morphine-pentobarbital. Two dosis of epinephrine (1 and 3/μg Kg) were injected before the administration of the insulin-glucose and thereafter every 30 minutes up to 4 hours.

The greatest reduction on the pressor responses to epinephrine occurred at the 60th and 90th minutes (from a control responses of 34 ± 4 and 56 ± 10 mm Hg to 8 ± 2 and 35 ± 9 respectively), with a serum K of $3{,}7\pm0{,}2$ mEq ($4{,}9\pm0{,}2$ control). However, the lowest level of serum K, $3{,}5\pm0{,}2$ mEq, was attained only at the 150 and 180th minutes when the pressure increases were almost normal. Thus, the blood pressure responses to epinephrine are normal two to three hours after the insulin-glucose infusion with serum K still low.

The number of the neurons in the intracardiac ganglia, according the technic elsewhere described (2), was subnormal in the rats chronic Chagas's disease. This fact suggests that the greater bradycardia produced in these rats by the vagus stimulation and acetylcholine injection can be due to a hypersensibilization determined by partial denervation of the parasympathetic nervous system.

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Shock experimental por hemorragia. S. MANCINELLI, E. VIVALDI Y G. PINARDI. *(Depto. Fisiopatología, Univ. Concepción, Concepción, Chile).*

Se indujo shock experimental por hemorragia mediante la técnica de Lamson y De Turk, modificada por nosotros.

Un grupo de perros fué sometido a shock reversible y otro a shock irreversible. En ambos grupos de animales se estudiaron las va-

riaciones de la frecuencia cardíaca y respiratoria, volumen minuto, presión arterial y venosa, resistencia periférica y pH sanguíneo.

En los animales que presentaron shock reversible la reinfusión total se acompaña de la normalización de los valores de pH sanguíneo, en tanto que en los perros que fallecieron, la acidosis persistió y aumentó, a pesar de la reinfusión total de sangre.

Modificaciones del pH en shock experimental por hemorragia. S. MANCINELLI, S. VIVALDI, G. PINARDI Y H. SALAZAR. *(Depto. Fisiopatología, Esc. Medicina, Univ. Concepción, Chile).*

Influence of intense or prolonged electric stimulation on systolic energy. H. MAZZELLA. *(Cát. Fisiopatología, Univ. de los Andes, Mérida, Venezuela).*

A study has been made on the action of electric stimulation, intense or prolonged, on the amplitude of the systole of the ventricle of the *Bufo arenarum* H.

Unipolar stimulation provoked systoles which were either larger or smaller than the control ones, depending on the polarity of the electrodes.

When the ventricle was stimulated with great asymmetry of electrodes, a greater systolic response was observed, which is explained in terms of the apparent inversion of the polar effect classically described.

The modifications in systolic amplitude seem to be partially due to intramyocardiac hormones depending on the sympathetic and also, in some measure, due to electrotonic effects produced by the stimulation.

The potentiation of the Bowdicht staircase seems to be provoked by a mechanism which is different from that of the excitation with high voltage-long duration pulse.

A technique for Starling's preparation carried out in guinea-pig. H. MEDINA. *(Inst. de Bioquímica Univ. Paraná, and Fac. Medicina, Univ. Paraná, Caixa Postal 939, Curitiba, Paraná, Brasil).*

During the experimental work which has been carried out in this laboratory on the effect of drugs on the oxidative phosphorylation of heart sarcosomes, it was necessary to study what would be the effect of such drugs as some synthetic antihistaminic (phenothiazinic compounds) on the cardiac work, since it was already determined that they acted on heart sarcosomes by inhibiting oxidative phosphorylation.

The preparation of Jerusalem and Starling (1) as well as Cruickshank's (2) modifications and the adaptation for rat, were the basis for the method studied on guinea-pig which is presented in this communication. Three main problems were solved in order to make it possible: 1) The artificial respiration with controllable vital capacity; 2) A suitable amount of blood to be obtained from the smallest number of animals; 3) The direct cannulation of the aorta.

The first problem was solved by the use of an injector of air built up with aid of a 20 ml syringe and a phonograph motor linked to a rheostat. Control of the vital capacity was done by changing the velocity of the air input into the tracheal cannula as well as the diameter of a tube which operated as a valve for the regulation of the injected air. The circulation of the air into the lungs in both directions was possible because of a set of valves adapted to the circuit.

The administration of gases like oxygen and anesthetics was done through the valve which allowed the aspiration of gas through the syringe.

Blood was obtained by the cannulation of the carotid artery of a second guinea-pig previously heparinized. A regular amount of blood was obtained after an intravenous and slow injection of small amounts of an isotonic solution of polyvinylpyrrolidone.

Blood circulation through the innominate artery and the superior vena cava supplied the heart with an amount of blood enough to maintain it in perfect conditions of function during the cannulation of both aorta and the inferior vena cava.

The circulation circuit followed the general lines of Starling's scheme. It was assembled with a resistance and a thermostat which controlled the temperature of a water-bath used to warm the container of the circulating blood. The entry of blood in the inferior vena cava was regulated by a pressure regulator which also worked as a peripheric resistance

for the blood when it went in the direction of the container. The innominate artery can eventually receive the cannula of a manometer.

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Influencia de la tiroxina sobre la liberación de substancia adrenalino-símil y sobre la cardioestimulación inducidas por la acetilcolina. S. MIDDLETON Y F. ALVARADO. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

Diversos autores han demostrado que la inyección intracoronaria de acetilcolina en el corazón atropinizado de mamífero, induce liberación de una substancia adrenalinosímil. Se sabe, por otra parte, que la administración prolongada de tiroxina aumenta las catecolaminas cardíacas y pareció de interés investigar la liberación de substancia adrenalinosímil en corazones de gatos "tiroxinizados".

Gatos adultos se inyectaron subcutáneamente con tiroxina en dosis diarias promedio de 0.4 mg/kg de peso corporal, durante 50 días; el peso de los diversos animales disminuyó, durante el período de "tiroxinización", entre 27 % y 38 %. La acción de la acetilcolina intracoronaria se estudió en el corazón aislado, perfundido con solución Tyrode y atropinizado. La liberación de substancia adrenalinosímil se estimó por la disminución del tono del ciego rectal (pollo), producida por los perfusados cardíacos.

La actividad adrenalinosímil de los perfusados equivalió a una concentración promedio de adrenalina de 4.1 x 10^{-9} en 7 corazones "tiroxinizados" y de 1 x 10^{-9} en 13 corazones control (t 2.59, $p < .025$).

La aumentada entrega de substancia adrenalinosímil en corazones "tiroxinizados" podría ser responsable del mayor efecto inotropo positivo que en ellos ejerce la acetilcolina, descrito por otros y que se observó también en nuestros experimentos.

Effect of hypotension produced by bleeding upon the EEG in rabbits. C. MUÑOZ AND L. GOLDSTEIN. *(Department of Pharmacology, Emory University, Atlanta, Ga., USA).*

The effects of blood pressure (BP) changes on the EEG were quantitatively studied on curarized rabbits under artificial respiration, by measuring the voltage output (electrogenesis) with the Electronic Integrator of Drohocki. One ml of blood per 100 g of body weight was removed through the femoral artery in 5 minutes. Ten minutes later, a volume of 6 % solution of Dextran equivalent to the removed blood was injected i.v. in 5 minutes. The same procedure was repeated 2 or 3 times on each animal. Changes in blood pressure and in the hematocrit volume were also determined.

During and immediately after bleeding an increase in electrogenesis coincident with the decrease in BP were constantly observed. The injection of Dextran restored both BP and electrogenesis to the control levels. Similar, but more marked, changes were observed during the second bleeding. When Dextran was injected simultaneously with bleeding, both BP and electrogenesis were maintained at the control levels, even when reduction of hematocrit volume to values about 1/3 of the initial were obtained.

Neither spinal cord section at C2, nor denervation of the carotid sinuses or section of both vagi nerves in the neck, prevented the electrogenesis changes induced by bleeding or the recovery with Dextran. BP as well as electrogenesis changes were considerably enhanced on animals with denervation of carotid sinuses.

Acción de la tripsina sobre el efecto "CRF" de la vasopresina. M. N. MUNTAABSKI. *(Cát. Farmacología Experimental, Fac. Farmacia y Bioquímica, Buenos Aires, Argentina).*

Hipotermia y circulación en el sapo. A. C. NACIMIENTO. *(Dept. Physiol., State Univ. New York, N. Y., USA).*

En el sapo intacto y no anestesiado sometido a una hipotermia en condiciones uniformes, se observó un descenso progresivo de la presión arterial, de la frecuencia cardíaca, y de la amplitud del efecto presor de la adrenalina intravenosa.

Tratando de determinar los posibles mecanismos de la modificación en la respuesta a la adrenalina, se estudiaron sus efectos en el corazón aislado y perfundido, y en el lecho vascular perfundido de las patas posteriores del sapo, a diferentes temperaturas.

El descenso progresivo de la temperatura del líquido de perfusión no modificó prácticamente la acción inotrópica positiva de la adrenalina en el corazón perfundido. En cambio, el efecto vasoconstrictor en el lecho vascular perfundido en las mismas condiciones, disminuyó considerablemente.

Respirómetro sencillo para animales pequeños. A. G. NEHME, Y L. BARDISA. *(Depto. Farmacología, Esc. Medicina, Univ. Concepción, Concepción, Chile).*

En base a las indicaciones de Scholander y otros autores construimos un equipo de respirómetros para trabajar con ratones blancos. Consta fundamentalmente, cada unidad de una cámara hermética en la cual se coloca el animal, comunicada con un manómetro de agua, un frasco lavador con KOH y un sistema simple para hacer circular el aire por todo el sistema.

En estas condiciones es fácil estudiar el efecto de fármacos en el consumo de oxígeno de los animales, tomando las debidas precauciones respecto a la temperatura y presión ambientales, expresando los resultados en microlitros de oxígeno por gramo por minuto en condiciones normales.

Método para el registro continuo de la frecuencia cardíaca fetal durante el parto. J. PANTLE Y J. J. POSEIRO. *(Serv. Fisiología Obstétrica, Fac. Medicina, Montevideo, Uruguay).*

Electrolytic method for preparation of pure oxygen for physiological purposes. J. V. PEREIRA LEITE. *(Dept. Fisiología, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

The introduction of accurate volume of oxygen in closed circuit respiration calorimeters presents some difficulties.

The electrolysis of a solution of Copper Sulphate with Platinum anode and Copper cathode is a very efficient way to obtain pure O^2 in precise amounts for respiration calorimetry of small animals.

The advantages of the use of Copper and platinum for electrodes have been discussed as well as precautions taken in order to assure uniform performance of the electrolytic cell.

Details of construction of the electrolytic cell were shown and also a chart of its performance at different levels of O^2 output.

The electrolytic cell here presented is a basic component for automatic measurement of O^2 consumption.

Efectos electro-cardiográficos provocados experimentalmente por el estreptococo hemolítico y sus toxinas. P. F. PIERONI Y G. HALSBAND. *(Fac. Farmacia y Bioquímica, Univ. Buenos Aires, Argentina).*

En el corazón de sapo (*Bufo arenarum H*) perfundido *in situ*, la inyección de cultivo de estreptococo hemolítico grupo A, provoca bloqueo aurículo-ventricular irreversible; la estreptolisina "O" paro sistólico ventricular reversible.

Por calentamiento, el cultivo y la estreptolisina "O" se tornan ineficaces. Electrocardiográficamente se comprueban los efectos en sapo.

En el conejo, la inyección intraperitoneal de cultivos de estreptococo, determina, entre otras manifestaciones, alteraciones en las ondas P_1 y T_1 en el segmento ST.

Con la estreptoquinasa y la estreptodornasa no se comprueban efectos en sapo y en conejo a las dosis empleadas.

Action of anoxia on the myocardium and behaviour of K^+ and Ca^{++} ions. A. PONCE ZUMINO, E. RUIZ PETRICH, H. CABALLERO Y J. R. E. SUÁREZ. *(Dpto. Fisiología, Fac. Medicina, Univ. Nac. Cuyo, Mendoza, Argentina).*

Two series of experiments with anoxia on the isolated cat heart were carried out, according to Langendorff. A series was perfused with Tyrode with 3.5 mEq/l of K^+ and 4 mEq/l of Ca^{++} and, in the other series, with

the same Tyrode to which had been added 10 % of washed bovine erythrocytes.

The action of anoxia on the mechanical and electrical activity of the heart, of K^+ and Ca^{++} balance and the variations of the coronary flow, was studied.

Relations were found in both series among the modifications of the ionic balance, the mechanical activity, some aspects of the electrical activity and the appearance of arrhythmias.

In those hearts perfused with Tyrode only, anoxia induced a depression of the chrono and inothropic properties with a positive balance of K^+ and a negative one of Ca^{++}. On the other hand in the hearts perfused with Tyrode with erythrocytes, it induced a stimulation of these properties with a negative balance of K^+ and a positive balance of Ca^{++}.

The coronary flow increased in both series, the increase being earlier and greater with Tyrode alone.

The recuperation of the heart perfused with red cells, after anoxia, was total, a recuperation which was not obtained in any of those perfused with Tyrode alone.

Influencia de las contracciones uterinas sobre la frecuencia cardíaca fetal. J. J. POSEIRO, M. A. ZAMBRANA, G. ARELLANO, N. WIQUIST Y W. FILLER. *(Serv. Fisiología Obstétrica, Fac. Medicina, Montevideo, Uruguay).*

Presión portal. Modificaciones provocadas por la hemorragia y la reinfusión de sangre. Estudios experimentales en perros. J. SANHUEZA, S. MANCINELLI, J. CHIANG-CHAU Y E. VIVALDI. *(Depto. Fisiopatología, Univ. Concepción, Concepción, Chile).*

Se estudia la presión portal y las modificaciones provocadas por la hemorragia y la reinfusión de sangre.

La hipotensión arterial provocada por la hemorragia se acompaña de disminución de la presión portal, que es tardía y no proporcional. La hemorragia controlada a fin de reducir la presión arterial a cifras de 70 mm Hg, provoca una disminución de la presión portal que guarda relación con la cuantía de la hemorragia.

La disminución de la presión portal por hemorragia no guarda relación con cambios de la presión venosa central y auricular derecha.

El aumento selectivo del flujo sanguíneo por la vena porta, sin modificación de la volemia ni de la presión arterial sistémica, se acompañó de aumento de la presión portal.

La reinfusión de sangre provoca aumento de la presión portal sobre los valores observados previamente a la hemorragia.

Ascorbic acid and hemorrhagic shock in dogs. I. Changes in plasma and in whole blood. J. A. SANTOMÉ AND O. A. GÓMEZ. *(Labor. Patología y Cirugía Experimental, Fac. Medicina, Buenos Aires, Argentina).*

The ascorbic acid content of whole blood, plasma and cells of the dog was studied during experimental hypotensive hemorrhage lasting for 2½ hs.

Whole blood and plasma ascorbic acid showed a highly significant increase 30, 60 and 120 min post hemorrhage.

Blood cells ascorbic acid, on the contrary, showed a highly significant descent, 60 and 120 min post hemorrhage.

Reinfusion of blood did not restore the ascorbic acid content of whole blood, plasma and cells to normal levels, even when prolonged for 5½ hs.

La participación del ventrículo derecho en la dinámica cardíaca. A. C. TAQUINI, M. E. BELLIZZI Y A. J. BOLLINI. *(Centro Investigaciones Cardiológicas, Univ. Buenos Aires, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Se midieron las presiones y el V. M. en perros en condiciones normales bajo anestesia y después del reemplazo de la pared del ventrículo derecho por una lámina de plástico. Las operaciones fueron realizadas con circulación extracorpórea.

El reemplazo del V. D. no modificó significativamente la cifra de presión sistólica; la presión diastólica mostró un incremento moderado; la curva de presión no cambió significativamente.

Se estudian y discuten las variaciones de

la presión intraventricular derecha siguiendo a la obstrucción parcial de la arteria pulmonar de la aorta, y antes y después de la denervación simpática.

Los resultados permiten concluir que la sístole del ventrículo izquierdo es un factor importante en la actividad mecánica del ventrículo derecho.

Destrucción del sistema nervioso central en perros con hipertensión nefrógena. A. C. TAQUINI (h.), J. D. FERMOSO Y A. C. TAQUINI. *(Centro Investigaciones Cardiológicas, Univ. Buenos Aires, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

En perros normales y con hipertensión de Goldblatt se estudiaron las variaciones hemodinámicas producidas por la destrucción del sistema nervioso central.

Previa anestesia con Pentobarbital 35 mg/kg de peso endovenoso se realizó craneotomía y extirpación del cerebro seguida de destrucción medular pasando una varilla de acero por el conducto raquídeo el que fué abordado por laminectomía caudal.

En todos los animales se midió: 1. volumen minuto por el método de Fick; 2. presión arterial sistémica, presión pulmonar y presión auricular derecha con Statham straingauge y electromanómetro; 3. volemia con azul de Evans método de Gregersen. Todas estas determinaciones se realizaron antes y después de la destrucción del sistema nervioso central.

Se discuten los resultados obtenidos en ambos grupos.

Punção biópsia do coração. N. VALLE-SOUBIHE E W. A. HADLER. *(Dep. Morfologia, Fac. Medicina Rib. Prêto, Univ. São Paulo, Ribeirão Prêto, SP, Brasil).*

El volumen minuto en la hipertensión esencial. H. V. VALIMITJANA, J. ARAMENDIA Y A. C. TAQUINI (h.). *(Centro Investigaciones Cardiológicas, Univ. Buenos Aires, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

En un grupo de enfermos con hipertensión esencial se estudiaron las variaciones del volumen minuto. Previamente, éstos fueron sometidos a los siguientes exámenes para descartar hipertensiones secundarias: 1. Aortografía; 2. Catecolaminas y 3. Estudios de la función renal.

Una semana antes de la determinación del volumen minuto se efectuaron dos consumos de oxígeno con el objeto de asegurar que los enfermos se encontraban en idénticas condiciones el día de la determinación. En los casos que esto no ocurrió se descartó la prueba.

El volumen minuto fué determinado por el método de Fick o el de Stewart Hamilton utilizando azul de Evans. En estos últimos ade más se midió consumo de oxígeno.

Los resultados en los primeros ocho experimentos muestran el volumen minuto en los límites altos.

Transporte de electrólitos por la circulación pulmonar estudiado en la preparación corazón-pulmón en el perro. D. YUDILEVICH. *(Inst. Física Matemática, Univ. Chile, Santiago y Dept. Physiol., Univ. Tennessee, Memphis, Tenn. USA).*

Curvas de dilución de varios indicadores radiactivos obtenidos simultáneamente permiten el estudio del movimiento de substancias plasmáticas entre los compartimientos intra y extravascular. Se presentarán los resultados obtenidos con este método con $^{42}K^{+}$, $^{24}Na^{+}$, $^{131}I^{-}$, $^{59}Fe^{+++}$ y $R^{131}ISA$, en la circulación pulmonar del perro estudiada en la preparación corazón-pulmón. $R^{131}ISA$ se usa como referencia de un indicador de distribución exclusivamente intravascular; de estas curvas de dilución se calcula (Método de Stewart-Hamilton) flujo de sangre y volumen del sistema y se propone análisis de ellas que permite estimar aproximadamente el flujo coronario de la preparación.

Los resultados indican que, en una sola circulación a través del pulmón, en las condiciones estudiadas, una fracción de la dosis de electrólitos radiactivos inyectada sale del espacio intravascular y parcialmente regresa a él.

Este hecho produce cambios en las curvas de dilución que dificultan la correcta medida del flujo cardíaco y del volumen del sistema.

Mediante el uso de un computador análogo electrónico se intenta simular las curvas de dilución de $R^{131}ISA$ y estimar la influen-

cia de la recirculación coronaria en la parte descendente de la curva.

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DIGESTIÓN
DIGESTION

Absorção intestinal de tiamina. A. CARVALHO DA SILVA, R. C. DE ANGELIS E M. GRZEGORZEWSKA. *(Dpto. Fisiologia, Fac. Medicina São Paulo, Univ. São Paulo, Brasil).*

Factors which influence the synthesis and secretion of gastric pepsinogen. L. CHIANG CH., I. BRAVO, J. CHIANG CH. Y J. CONCHA. *(Depto. Fisiología, Univ. Concepción, Concepción, Chile).*

The levels of pepsinogen in the gastric juice and in the wall of the stomach were studied under basal conditions and also after the administration of adrenaline and of histamine.

It was found that the quantity of pepsinogen secreted by the stomach, as well as the pepsinogen stored in the gastric glands, increased after the administration of both these drugs.

Acción de la aminoguanidina sobre el estómago de la rata con píloro ligado. B. LOZZIO, L. BIEMPICA M. ROYER Y S. GORODISCH. *(Inst. Gastroenterología, Inst. Nac. Salud, Haedo, Buenos Aires, Argentina).*

Se ha estudiado la acción de la aminoguanidina (AG) sobre la secreción gástrica, en la rata con píloro ligado, durante cuatro horas. La AG produce solamente un moderado aumento de la secreción gástrica con gran incidencia de úlceras y equimosis en la zona glandular gástrica.

La histamina aumenta la cantidad de pepsinógeno hístico pero no produce cambios en los demás componentes de la secreción gástrica.

La administración de ambas drogas simultáneamente, no ocasiona modificación alguna, comparado con los testigos; hubo un cambio en la localización de las lesiones, y en este grupo predominaron las úlceras de la zona pavimentosa, con marcada disminución de las lesiones descriptas para la zona glandular.

Los resultados son analizados, relacionando los hallazgos bioquímicos e histológicos, con la actividad histaminolítica de cada porción del estómago. Siendo la AG el inhibidor más específico de la histaminasa, se presentan algunos datos sobre el posible mecanismo en la secreción gástrica. La inyección de aminoguanidina no sensibiliza a la rata, con la cantidad de histamina usada.

Changes on enzymatic activity of dog submaxillary saliva influenced by thyroid and adrenocortic hormones. J. A. OSORIO. *(Inst. Fisiología Experimental, Fac. Medicina, Pôrto Alegre, Brasil).*

Thyroidectomized or DDD-treated dogs to induce thyroid and controlled adrenocortical insuffiency were used. The submaxillary enzymatic activity of saliva was studied through its depressor effects and determined by means of blood pressure recordings.

Saliva of treated dogs (2 ml of 20 % saline) were injected on normal and sialadenectomized animals. Alterations were produced on initial pressor phase as well as on the depressor phase. No remarkable difference was found between these two groups.

Acción de la biliverdina sobre la eliminación biliar de biliverdina y bilirrubina en la rata. M. ROYER, E. A. RODRÍGUEZ-GARAY Y T. C. ARGERICH. *(Inst. Gastroenterología, Inst. Nac. Salud, Haedo, Buenos Aires, Argentina).*

Se estudió la eliminación biliar de biliverdina y bilirrubina por acción de la sobrecarga de biliverdina, por inyección intraperitoneal, en ratas con colédoco cateterizado. El aumento observado en la eliminación de ambos pigmentos, parece confirmar que la biliverdina es precursora de la bilirrubina.

Las determinaciones efectuadas antes y después de la inyección, comparadas con las del

lote de animales testigos, permiten establecer la capacidad del hígado de la rata para la eliminación de dichos pigmentos. Se discute el porcentaje de transformación observado y su relación con la naturaleza del solvente, la influencia de la anestesia, la dosis inyectada, el peso del animal y el volumen de las fracciones recogidas en distintos intervalos de tiempo.

Action of hydrochlorothyazide on gastric secretion in the rat. M. SCHRAIER· P. MAZURE· B. LOZZIO AND L. BIEMPICA. *(Inst. Gastroenterología, Inst. Nacional Salud, Haedo, Buenos Aires, Argentina).*

In pylorus-ligated rats, the hydrochlorothyazide inhibitory effect upon hydrochloric acid in basal and histamine stimulated gastric secretion was studied.

The pepsin and hydrochloric acid output was significantly reduced.

Effects of hypophysectomy on the amylase and trypsin activatable protease activities in the pancreatic juice and pancreatic acinar cell of rat. A. SESSO AND V. VALERI. *(Depto. Morfología, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

Amylase and trypsin activable protease (TAPase) activities were measured in the pancreatic juice of fasted hypophysectomized and control animals.

Amylase and TAPase activities, Desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were measured in pancreas homogenates of the same animals.

In the hypophysectomized rats, the following changes were observed in relation to pair-fed controls:

a) The amylase activity per cell in the organ (= amylase activity per μg of DNA), and the amylase activity per hour and per cell in the juice were reduced to about 10 %.

b) The TAPse activity per cell in the organ was reduced to 57 % and the TAPase activity per hour and per cell in the juice was reduced to about 31 %.

c) The RNA per cell in the organ was reduced to about 49 %.

The effect of the reduction of the food intake due to hypophysectomy was evaluated through the comparison between the pair-fed and the fed ad libitum controls: in the pair-fed controls, the amylase activity per cell in the organ was found to be significantly reduced: some reduction was observed in the amylase activity in the juice, but the difference did not attain the significance level. No differences were observed for the TAPase activity in the organ and in the juice, nor for the RNA in the organ.

The relation between the enzyme activity in the juice and in the organ was found to be constant in all the experimental groups.

Hypophysectomy did not appear to modify the rate of flow of the juice per cell and per unit of time.

It is concluded that hypophysectomy depresses more the amylase synthesis in the pancreatic cell than that of TAPse. However, the discharge of the enzyme in the juice maintains a constant relation with the actual enzyme content of the cell. The permeation of water from the cell to the lumen of the acini is not modified by hypophysectomy when expressed per cell unit; since hypophysectomy modifies the volume of the cell, it is also concluded that the permeation of water in fasting conditions is not correlated with the volume of the cytoplasm of the acinar cell.

ENDOCRINOLOGÍA
ENDOCRINOLOGY

Genital tract modifications in the diabetic female rat. R. F. BORGHELLI, I. J. SPINDLER AND V. G. FOGLIA. *(Inst. Fisiología, Fac. Medicina, Univ. Buenos Aires, Argentina).*

The female albino rat's sexual cycle was studied in diabetic and prediabetic states following partial pancreatectomy (95 %) by the daily observation of the vaginal smears and the periodical anatomical examination of the genital tract.

During the diabetic state a decrease in the monthly number of cycles was observed and a shortening of estrous phases, which may bring its disappearance. The general rate shows

exceedingly significant differences with regard to controls.

During the prediabetic state the average number of monthly estrous days shows exceedingly significant differences with regard to controls; but not the average number of monthly cycles, the difference of which has not statistical significance.

No anatomical alterations in the genital tract of albino rats in prediabetic state were found.

The influence of some hormonal factors on the glycogen content of the normal and intrasplenic ovary in the C57Bl mice. S. BRUZZONE. *(Inst. Química Fisiológica y Patológica, Esc. Medicina, Univ. Chile, Santiago, Chile).*

Previous unpublished experiments of this laboratory have shown that homogenates of normal and intrasplenic ovaries incubated in a fortified medium in the absence of added fluoride, do not glycolyze glucose. On the other hand, there is a gradual decrease of an endogenous substrate that appears to be glycogen with the concomitant liberation of ketopentoses and ketohexoses into the medium. These observations suggested that glycogen has an important rôle in the normal ovary and specially in the fast growing intrasplenic ovary. If this is the case, any hormonal disturbance interfering with the development of the splenic grafted ovary, or with the cyclic changes of the normal ovary would be paralleled by alterations in the glycogen content.

Adult castrate females bearing one intrasplenic ovary for 60 days, received 10 μg daily of 17 β-estradiol for 10 days. At the end of this period the average weight of the graft was 12.6 mg in contrast to 25 mg in the non-treated animals. This reduction in weight of the grafted ovary was accompanied by an increase in the glycogen content. While in the non-injected animal the average glycogen content was 190 μg per 100 mg fresh tissue, in the estradiol-injected animals was 440 μg/100 mg of fresh tissue.

Serum gonadotropin was injected in normal adult females, at the dose of 10 I.U. daily during 15 days. The average weight of the ovary of the non-injected animals was 3.9 mg, while in the treated ones, it increased to 15.3 mg. However the glycogen content was not appreciably different in treated and not treated animals, 394 and 344 μg/100 mg of wet tissue respectively.

Since estradiol administration inhibits the hypophysis gonadotrophic activity, one is tempted to postulate that this inhibition is responsible for the accumulation of glycogen in the grafted ovary under estradiol treatment. This glycogen accumulation would be due to interference with metabolic reactions leading to glycogen utilization. This is supported by our unpublished findings which indicate that grafted ovary phosphorylase from estradiol-treated animals require AMP for activation. The latter is not needed in the case of non-treated animals.

El desarrollo de los descendientes de ratas tratadas con distintos esteroides durante la preñez. G. E. BUR, E. MONTUORI Y A. KOROMPAI. *(Depto. Investigaciones, Lab. Dr. Gador, Buenos Aires, Argentina).*

Epitalamo y función tiroidea. J. M. FLORIT. *(Inst. Biología y Medicina Experimental, Lab. Neurobiología, Obligado 2490, Buenos Aires, Argentina).*

Se realizaron lesiones electrolíticas que destruyeron los gánglios de la habénula en ratas Wistar de 200 gramos, machos y hembras. Se estudió la función tiroidea en estos animales y la hipertrofia compensadora que sigue a una hemitiroidectomia.

Para valorar la función tiroidea se consideraron los siguientes parámetros: peso de la glándula, captación de I^{131} a las 24 horas, contenido de Iodo 127 en la glándula, iodo proteico, relación de conversión y relación iodo tiroideo/iodo sérico.

Prediabetes, diabetes y preñéz en la rata. V. G. FOGLIA, R. CHIERI Y G. DONADIO. *(Inst. Fisiología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Se han estudiado tres grupos de ratas: a) pancreatectomizadas 95 % y preñadas 6 veces en 36 semanas de estudio; b) testigos pancreatectomizadas 95 % que no fueron puestas a preñar y c) testigos laparotomizadas y preña-

das también en 6 oportunidades durante 36 semanas.

Las ratas de los grupos a) y c) fueron colocadas de a tres con un macho normal durante 5 días y luego separadas en jaulas individuales hasta el momento de la parición efectuándoseles glucemias periódicamente durante la preñez y en el post-parto; a las testigos del grupo b) se les efectuaron glucemias de control una vez por mes.

Las crías eran individualizadas por madre clasificadas por sexo y pesadas dentro de las 24 horas, sacrificándolas para estudio histopatológico del páncreas.

También se estudió la histopatología de la hipófisis y páncreas en las ratas de los tres grupos.

Se observó en las madres del grupo a): disminución de la fertilidad durante los períodos de prediabetes y diabetes que inclusive llega a la esterilidad en algunos casos; cuanto más prolongada es la prediabetes tanto mejor es el grado de fertilidad. Las recién nacidas de las ratas de este grupo presentan aumento del peso corporal; disminución del número de crías por camada, debido a la reducción del número de machos.

Los resultados de la histología se presentarán con la comunicación.

Acción de la cloropropamida sobre el período prediabético experimental de la rata. V. G. FOGLIA, R. YABO Y L. D'ANNA. *(Inst. Fisiología, Fac. Ciencias Médicas, Univ. Buenos Aires, Argentina).*

Para estudiar la influencia de la administración crónica de cloropropamida (clorobencenosulfonilpropilurea), sobre el período prediabético experimental de la rata, se administró esta droga, por vía bucal, a animales con pancreatectomía parcial del 80 %. La dosis aplicada diariamente fué de 100 mg por kilogramo de peso corporal.

Los animales se dividieron en varios lotes en los cuales se estudió: la tolerancia oral a la glucosa, la respuesta hiperglucémica alimenticia, la glucemia de ayuno y, al final del tratamiento, se hizo estudio histológico del resto de páncreas que no se había extirpado en la ectomía parcial.

Los resultados obtenidos se separan en dos fases; una, inmediata y transitoria, (un mes) de mejor tolerancia a los hidratos de carbono y glucemias de ayuno más bajas en el lote tratado con la droga; y otra, que sigue a la anterior (dos y tres meses) de menor tolerancia a los hidratos de carbono y glucemias de ayuno más altas. En cuanto a la duración del período prediabético fué semejante tanto en el lote tratado con la droga como en el testigo.

Todo esto hace pensar que la cloropropamida no provoca regeneración de células beta en la rata prediabética, tal cual lo hace, según algunos autores, en el perro.

β-Glucuronidase and glucuronidation activities in rats treated with tumorigenic doses of estrogens. F. FUENZALIDA AND R. CHUAQUI. *(Inst. Química Fisiológica y Patológica, Esc. Medicina, Univ. Chile, Santiago, Chile).*

Groups of castrated female rats were implanted subcutaneously with estrogen pellets of different composition in order to ascertain the changes in glucuronidase activity in liver and hypophysis, and p-nitrophenyl-glucuronide synthesis in liver.

An increase in β-glucuronidase activity was observed in the liver of animals sacrificed after 5 months of treatment with β-estradiolbenzoate.

An increase in β-glucuronidase activity was observed also in the hypophysis of animals after 11 months of treatment with β-estradiol, or 5 months with β-estradiol-benzoate, or 5 months with diethyl stilbestrol. The increments in glucuronidase activity showed positive correlation with the augmented weights of the hypophysis.

Glucuronide synthesis in the liver of animals treated with β-estradiol was not significantly different from the control group.

Radioactive phosphorus and fertility. E. A. D. HOLMBERG, A. PAVLOVSKY, E. ARINI, C. D. PASQUALINI AND S. L. RABASA. *(Sec. Leucemia Exper., Acad. Nac. Medicina, Buenos Aires, Argentina).*

The effect of P_{32} on the gonads of BALB mice was studied. The ovary is more sensitive to this agent than the testes since 40 μc induce

sterility in the female, while all the males surviving to any dose of P_{32} up to 205 μc remain fertile. These animals were checked for fertility before the injection of P_{32} and were given the opportunity of mating afterwards during periods of 5 to 12 months.

The histologic pattern of the ovaries is that of a generalized luteinization without primordial follicles. Testes show no alterations.

Females injected with P_{32} during pregnancy remained fertile after doses of 40 and 60 μc and needed 90 μc to become sterile, which represents more than twice the sterilizing dose for a non-pregnant female.

Progesterone at a dose of 2 mg per week during 4 weeks before P_{32}, and 2 weeks more afterwards, affords some protection but it is not so complete as that afforded by pregnancy

This same phenomenon is studied after X-ray irradiation.

Variations in serum and aqueous humor mucoproteins in the sexual cycle of the rat. A. B. HOUSSAY AND N. BLUMENKRANTZ. *(Centro de Endocrinología, Min. de Asist. Soc. y Sal. Pública, Godoy Cruz 1221, Buenos Aires, Argentina).*

The mucoprotein content in serum and aqueous humor was studied in 88 female rats at different times of the sexual cycle and in 30 male rats of the same age. The amount of mucoproteins was determined by the tryptophane method and that of total carbohydrates in the aqueous humor was assessed by the same method. The degree of polymerization of mucopolysaccharides was studied qualitatively by the toluidine blue microtest and by the phenomenon of rhythmic crystallization of the aqueous humor.

In serum the mucoprotein content was found to be higher in the rats in estrus than in those in diestrus and of intermediate values in metaestrus and proestrus. In males the serum mucoprotein showed values equal to those in females in diestrus.

In aqueous humor in rats in estrus it was found a lower mucoprotein content with a higher degree of polymerization and a rhythmic crystallization of the fern-like type. In rats in diestrus there is a higher mucoprotein content with a lower degree of polymerization and a rhythmic crystallization of the choral-fern mixed type. In proestrus and metestrus there are intermediate values. In males the mucoprotein values are similar to those in proestrus and metaestrus and the degree of polymerization and crystallization similar to diestrus.

These results show the occurrence of a cyclic variation in the amount and quality in serum and aqueous humor mucoproteins in the sexual cycle of the female rat. In males the values are similar to those in the female in diestrus. It is supposed that estrogens are responsible for these cyclic variations.

* This work was supported by a "Fight for Sight Grant-in-Aid of the National Council to Combat Blindness, Inc.", New York City.

Sexual difference in the concentration of I^{131} by the submaxillary glands in A_2G mice. A. B. HOUSSAY, F. R. ALBERTI, M. SLATOPOLSKY, E. THIERER AND J. M. ROSNER. *(Cent. de Endocrinología, Min. de Asist. Soc. y Sal. Púb., Godoy Cruz 1221, Buenos Aires, Argentina).*

The submaxillary and sublingual glands, but not the parotid glands of A_2G mice have the property of concentrating I^{131}.

There is a marked sexual difference in the I^{131} concentration by the submaxillary glands, this concentration being higher in males than in females, which coincides with the greater height of the epithelium of the alveolar ducts.

Castration of males decreases the I^{131} concentration when enough time has elapsed since operation to cause an atrophy of over 20 % of the submaxillary glands. Castration does not decrease the I^{131} concentration by the submaxillary glands in females.

The administration of testosterone propionate increases markedly the I^{131} concentration by the submaxillary glands in castrated mice. However, the administration of estradiol benzoate or dipropionate fails to alter this concentration.

There is an evident parallelism between the height of the epithelium of the alveolar ducts and the concentration of I^{131} by the submaxillary glands that would support the hypothesis of the role of this epithelium in the I^{131} concentration.

Action of pituitary hormones upon the estrogenic function of adrenal tumor of castrated mice. A. B. HOUSSAY, E. I. SAURER Y R. LUCINI. *(Centro de Endocrinología, Min. de Asist. Soc. y Sal. Púb., Godoy Cruz 1221, Buenos Aires, Argentina).*

In rats and mice, castration produces, after several months, adrenal tumors with estrogenic activity. One of us has demonstrated that hypophysectomy causes the disappearance of estrus, which is restored by the action of chorionic gonadotropins but not by adrenocorticotropin.

The irregular estrogenic cycle that exhibit the castrated C_3H mice and castrated Ce mice with adrenal nodular hyperplasia and adenomas, was studied. It was observed the effect of the administration of various hormones for 5 days.

It was thus obtained an intense stimulating action during the 5 days of hormone administration when chorionic or pituitary gonadotropin was used (FSH and prolactin). No stimulation was observed after administering LH, TSH, ACTH or neurohypophysial extracts.

An inhibitory effect was elicited with high doses of androgens but not with corticosteroids.

Morphological and functional recovery of the testes of the rat atrophied by the prolonged action of estradiol. R. IGLESIAS. *(Inst. Medicina Experimental, Irarrazaval 849, Santiago, Chile).*

It is well known that estradiol inhibits the gonadotrophic function of the pituitary gland. In recent work Patanelly and Nelson (First International Congress of Endocrinology, Copenhagen, 1960, Advance Abstracts of Short Communications, p. 529) have shown that in the rat 50 micrograms daily of estradiol administered by injections reduces the gonadotrophic activity of the rat's hypophyses to 40 % after one week of treatment and that if this is prolonged, the gonadotrophins are reduced to zero. It is likewise well known that in the rat prolonged treatment with estradiol produces pituitary tumors, marked atrophy of the testes and concomitant sterility. If in these experiments the treatment with estradiol is suspended, the size of the testes is re-established and the lost fertility is recovered (See Review of Emmens and Parks in Vitamins and Hormones, Vol. 5, p. 233, 1947).

In the course of experiments designed for the purpose of studying the delayed after effects of a prolonged treatment with estradiol in the rat, we have confirmed the results mentioned above. Furthermore, we have observed that the seminal vesicles of the animals recovered after the treatment with estradiol are larger than those of normal males.

Young adult male rats of the AxC strain have been subcutaneously implanted with a half pellet of pure estradiol weighing 15 mg. In this manner each animal is provided with about 50 micrograms daily of estradiol. All the animals were treated during 4 months. One group was killed immediately after this treatment. The other was left to survive one year after the treatment was suspended. In the first group, great atrophy of testes was observed, the weight being one tenth the normal weight. Atrophy of seminal vesicles and of the prostate and large tumors of the pituitary gland (200 mg) were also observed. In the second group which survived one year after the continued four month treatment with estradiol, the weight of the testes became normal; the weight of the seminal vesicles was greater than in the controls. The average weight of the pituitary gland was 32 mg, that is, some three times greater than in normal animals. The quantity and motility of the sperm cells were seemingly normal. Out of four animals tested, two proved to be fertile.

This study was aided by a grant from the Population Council, New York, New York.

Adrenal function in male rats with bilateral lesions in the preoptic area. C. P. LANTOS AND J. H. TRAMEZZANI. *(Inst. Biología y Medicina Experimental, Lab. Neurobiología, Obligado 2490, Buenos Aires, Argentina).*

Adrenal function in male rats with bilateral lesions in the preoptic area was studied. The following parameters were considered: a) gland weight; b) total corticoids per gland (corticosterone plus cortisol); c) total corticoids

in peripheral blood (corticosterone plus cortisol).

One month after lesion, the gland weight ($P < 0.001$) and corticoid content in peripheral blood ($0.05 < P < 0.01$) of operated animals increased as compared with their controls. Corticoid content of total gland, as referred to weight of animal, was higher ($0.05 < P < 0.01$) in operated than in control rats, but corticoid content per weight unit of gland remained unchanged. Results are discussed.

Endocrine glands and secretion of I^{131} by the submaxillary gland of C_3H mice. J. L. LLACH. *(Labor. Neurobiología, Inst. Biología y Medicina Experimental, Obligado 2490, Buenos Aires, Argentina).*

The effect of castration, adrenalectomy and the administration of testosterone, estradiol, corticosterone and aldosterone on the concentration of iodine by the submaxillary gland of male and female C_3H mice, has been studied. The ratio between iodine 131 by mg of submaxillary gland and iodine 131 by mg of blood (SM-B ratio) was taken as an index of the submaxillary gland's ability to concentrate iodine.

Castration increased the SM-B ratio in the male and did not modify it in the female, so that in the castrated animals the sexual difference of the SM-B ratio became more evident. The administration of testosterone to the castrated male restored the changes induced by castration. Estradiol in the castrated female diminished the SM-B ratio in close relation with the given doses.

Adrenalectomy in both sexes determined a raise in the SM-B ratio. In the adrenalectomized animals, corticosterone diminished the SM-B ratio to the normal values and aldosterone had no action. The administration of corticosterone to normal mice diminished in the male the uptake of iodine 131 by the submaxillary gland and increased it in the female. Aldosterone enhanced the SM-B ratio in both sexes.

It is thought that these changes occur through two mechanisms: 1) structural modifications of the submaxillary gland, and 2) functional alterations, mainly in the salivary secretion.

Hialuronidase e a resposta do tecido de rato a pequenas concentrações de insulina. E. MAGALHÃES. *(Inst. Fisiología Experimental, Fac. Med. de Pôrto Alegre, Univ. Rio Grande do Sul, Pôrto Alegre, RGS, Brasil).*

A hialuronidase na concentração de 10 ou 15 unidades redutoras de turvação por ml, adicionadas simultâneamente com a insulina "in vitro", produz um aumento significante da quantidade de CO_2 despendido pelo tecido adiposo do epidídimo de rato durante a síntese de lipídios a partir de glicose sob a influência de 20 ou 50 microunidades de insulina por ml. A hialuronidase sòzinha não produz nenhum efeito.

Lipogenesis and cholesterogenesis in the liver of the totally pancreatectomized rat. R. H. MIGLIORINI AND I. L. CHAIKOFF *. *(Depto. Fisiología, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil and Dept. Physiology, Univ. California, Berkeley, Cal., U.S.A.).*

Female Long-Evans rats weighing 150-170 g were used. After a 17 hours fast more than 99 % of the pancreas was removed under ether anesthesia by the procedure of Scow (1). The animals were given in the first 3 postoperative days increasing amounts of insulin and food, so that by the fourth day each rat received twice daily 9 U of regular insulin subcutaneously and 4.5 g of food to which pancreatin was added. The diet administered contained 20 per cent casein, 67 per cent dextrin, 8 per cent fat and adequate amounts of salts and vitamins. There was an interval of about 8 hours between meals. Control rats, which were deprived of the pancreas lying along the bile duct and between this duct and the first portion of the duodenum, were similarly treated with the exception of the administration of insulin.

The animals were maintained on this regimen until the 10-12th postoperative day, when the insulin treatment of the depancreatized rats was interrupted. They were sacrificed for study 18 and 30 hours after the last injection of insulin. Their livers were rapidly removed, sliced and incubated in appropriate

buffer solution containing either acetate-1-C^{14} or glucose-E-C^{14}. The incorporation of these substrates into fatty acids and cholesterol, as well as their conversion to CO^2, was determined.

There was a pronounced decrease in the incorporation of acetate and glucose into fatty acids 18 hours after suspension of insulin. At the end of 30 hours the liver slices had almost completely lost the capacity to form fatty acids from added glucose and acetate. There was a concomitant fall in the percentage of C^{14} from glucose recovered as CO^2.

The incorporation of acetate into liver cholesterol showed a two to seven-fold increase at the 18 hours interval, when compared to the controls. This increase was much less pronounced at the end of 30 hours.

The results are discussed and compared to the findings in other types of experimental diabetes.

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Action of purified gonadotrophins on gonadal differentiation in the chick embryo *. R. Narbaitz **. *(Inst. Anatomía General y Embriología, Fac. Medicina, Univ. Buenos Aires, Argentina).*

Although many workers have demonstrated that the hypophysis does not interfere on gonadal differentiation, this does not exclude the possibility of producing changes in this process through the action of foreign gonadotrophins. Gonadal differentiation is believed to depend on the balance between secondary organizers whose chemical nature remains unknown.

The production of different degrees of sex inversion through the injection of sexual steroids, gives indirect evidence supporting the view that normal organizers may be chemically related to them, but actual biochemical demonstration of this assert is still lacking.

Believing that the injection of embryos with substances which may interfere in a specific way with sex steroid production, such as gonadotrophins, may give additional evidence, we injected more than 200 chick embryos with doses varying between 20 and 150 μg of FSH and LH kindly supplied by N.I.H. (Bethesda) using different schemes and sites of injection.

High doses produced interesting morphological changes, the most important of which were the production, in the testis, of new sex cords of typically masculin structure through the action of FSH, and the exaggeration of normal vacuolization of ovarian medulla through the action of LH.

These changes may be interpreted as a direct action of gonadotrophins. Sex ratio was normal and no evident sign of sex inversion or intersexual transformation was observed.

These results may be interpreted in two ways: either gonadal differentiation does not depend on a balance between androgens and estrogens or gonadotrophins do not affect the production of these substances in the early embryo. In the light of other kind of evidence the author believes the first supposition is more probably true.

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** With the technical assistance of Beatriz Sucharewicz.

Estudo da contratilidade uterina na coelha nas diversas fases do ciclo grávido-puerperal. C. E. Negreiros de Paiva. *(Depto. Fisiologia, Fac. Medicina, Rib. Prêto, Univ. São Paulo, Ribeirão Prêto, SP, Brasil).*

Effect of purified FSH upon the male gonad of *Pleurodema cinerea* after recent metamorphosis. A. Pisanó and M. H. Burgos. *(Inst. Biología Marina, Mar del Plata, Argentina; Inst. Histología y Embriología, Fac. de Ciencias Médicas, Univ. Nacional de Cuyo, Mendoza, Argentina).*

Ten injections of 0.05 mg of purified FSH in 0.1 ml of frog saline solution were administered every other day to *Pleurodema cinerea* recently metamorphosed (differentiated race). This hormone produced a maturation wave of germinal cells in the genetic males.

While in the controls only cords of germinal cells remain, in those hormone-treated animals large ampoules appear with the complete spermatogenic wave, from spermatogonia to spermatozoa.

This finding shows purified FSH as the factor capable of producing a complete sperma-

togenic wave in this differentiated race. The interstitial tissue maintained the embrionic characteristic.

Acción de la hormona folículo estimulante (FSH)sobre el testículo recién diferenciado del *Bufo arenarum* Hensel. N. Z. PIZARRO Y M. H. BURGOS. *(Inst. Histología y Embriología, Fac. Ciencias Médicas, Univ. Nac. de Cuyo, Mendoza, Argentina).*

La inyección de FSH en solución fisiológica en dosis de 0.05 mg durante 20 días inyectados día por medio por vía intraperitoneal provoca, en sapos machos recién diferenciados, la transformación de los cordones o conglomerados celulares testiculares en ampollas seminíferas. Dentro de tales ampollas las gonias, con el aspecto característico de las espermatogonias maduras (núcleo polilobulado y múltiples nucleolos) aparecen rodeadas por típicas células foliculares. Los conglomerados celulares aparentemente se ahuecan por la incorporación de líquido que se acumula en su interior formando una cavidad central. Estas ampollas al dilatarse provocan la separación de las espermatogonias entre sí que aparecen acompañadas por células foliculares típicas con nucleolos prominentes. Al mismo tiempo se hace evidente la formación de una membrana basal, delicada, alrededor de las ampollas. Las células del intersticio se mantienen con los mismos caracteres indiferenciados que los controles.

En ninguno de los animales de esta raza indiferenciada, inyectados con FSH se logró provocar una onda espermatogénica completa, no pasando, el aparente estímulo, de madurar a las espermatogonias y células foliculares.

Se discute el mecanismo de formación de las ampollas seminíferas y la probable intervención directa del FSH como inductor.

Antidiuretic hormone during water deprivation in the rat. S. L. RABASA AND F. BERGMANN. *(Inst. Investigaciones Médicas, Rosario, Argentina).*

Plasma obtained after ether anesthesia from rats deprived of water during 1-4 days shows more antidiuretic activity than normals and has, moreover, a steeper dose-response curve.

Acetone desiccated extracts from the posterior lobes of animals deprived of water during one day show the reverse phenomenon; that is, a dose response curve with a lower slope, while the total activity per unit weight is approximately the same as that of normals. The assays of these substances were performed in rats by the intraperitoneal and intravenous routes and in toads with the same results. The hypothesis is advanced that during water deprivation a more active type of antidiuretic hormone is at work.

Respuesta hipofiso-suprarrenal de la rata al tratamiento crónico con tiroxina. D. RAMÍREZ, E. GALLARDO-LOYOLA Y M. BRAVO. *(Cát. Fisiopatología, Esc. Medicina, Univ. Chile, Santiago, Chile).*

En ratas se determinó la relación entre la dosis de tiroxina y la respuesta del sistema hipófiso-suprarrenal. La tiroxina se administró diariamente en inyección subcutánea en dosis de 0.1, 0.2 y 0.5 mg/100 g de peso corporal durante 12 días; 24 horas después se sacrificaron los animales bajo Nembutal. Las suprarrenales de las ratas tratadas presentaron hipertrofia y disminución de su concentración de ácido ascórbico, aproximadamente proporcionales a la dosis de tiroxina. La producción de corticoides *in vitro* por cortes de suprarrenales, expresada por unidad de peso, fué de igual magnitud en los animales tratados con tiroxina y en los controles. La concentración de actividad adrenocortocotrófica de los extractos hipofisarios (medida por su capacidad de vaciar el ácido ascórbico de suprarrenales de ratas de ensayo pretratadas con prednisolona) de las ratas tratadas con tiroxina fué significativamente mayor que la de los controles.

Los resultados permiten plantear que, en ratas, el tratamiento prolongado con diferentes dosis de tiroxina estimula tanto la síntesis como la liberación de ACTH y que las modificaciones del peso y ácido ascórbico suprarrenal observadas (probablemente debidas a una mayor liberación de ACTH u otros factores hipofisarios) tienen umbrales de respuesta aproximadamente similares.

Potencialização da ação da insulina pela hialuronidase. P. RIET CORREA E E. MAGALHÃES. *(Inst. Fisiologia Experimental, Fac. Medicina de Pôrto Alegre, Univ. Rio Grande do Sul, Pôrto Alegre, RGS, Brasil).*

A administração de insulina e hialuronidase por via intravenosa, em cães, produziu maior abaixamento da glicemia do que quando a insulina era injetada isoladamente, sendo a diferença estatisticamente significativa sòmente aos 10 e 20 minutos.

Discute-se o possível mecanismo pelo qual a hialuronidase produz êste efeito.

Efeito de adrenalina sôbre a sensibilidade do tecido adiposo à insulina "in vitro". P. RIET CORREA E E. MAGALHÃES. *(Inst. Fisiologia Experimental, Fac. Medicina de Pôrto Alegre, Univ. Rio Grande do Sul, Pôrto Alegre, RGS, Brasil).*

1. A adrenalina adicionada ao tecido adiposo do epidídimo de rato "in vitro" na concentração de 1 micrograma por ml causou um aumento significativo do CO_2 libertado a partir da glicose: também acentuou o efeito de 20, 50 ou 100 microunidades por ml de insulina adicionada simultâneamente.

2. Quando a adrenalina era injetada na dose de 0.02 mg/100 g, uma hora antes da remoção do tecido, não se observou nenhum efeito sôbre as trocas gasosas. A ação da adrenalina sôbre a síntese de lipídios a partir da glicose no tecido adiposo do epidídimo "in vivo" parece ser transitória, e o seu significado no balanço metabólico não pôde ser estabelecido.

Hyperglycemia caused by prolonged administration of high doses of tolbutamide to turtles. P. RIET CORRÊA AND MARIA MARQUES. *(Inst. Fisiologia Experimental, Fac. Medicina, Pôrto Alegre, RGS, Brasil).*

Chrysemys d'Orbignyi turtles received daily by intraperitoneal way, a solution of sodium tolbutamide in saline. A dosis of 100 mg/kg was used in the first nine days, being increased to 200 mg/kg in the following fourteen days, and to 500 mg/kg in the last six days. Controls received only saline solution.

Dosis of 100 and 200 mg/kg did not modify glycemia, but in the 500 mg/kg dosis the glycemic values went up to 341 mg % in treated animals, compared to 57 mg % found in control group.

Tolbutamide, in high dosis, showed to produce hyperglycemia in this animal species.

The action of oral hypoglycemic compounds on the onset and recovery of experimental diabetes. R. R. RODRÍGUEZ, F. R. A. DUMM, J. J. GAGLIARDINO AND H. O. BUSCHIAZZO. *(Inst. Fisiología, Fac. Medicina, Univ. La Plata, La Plata, Argentina).*

White ovariectomized subtotally depancreatized rats fed "ad libitum" were treated during seven months with 30 mg per rat daily of 2259 R.P. Afterwards, the administration was discontinued and the animals were followed for another six months. Blood and urine sugar were determined monthly in order to observe the onset of diabetes. Forty two animals were studied, half of them treated and the others remained as untreated controls.

During the first period the incidence of diabetes was slightly less in the treated rats (18 % compared with 24 % in the controls; data not statistically significant).

In the next six months, without treatment, the incidence of diabetes rose to 69 % in the experimental animals and to 57 % in the controls. This difference was neither significant.

In another experiment, 24 alloxan diabetic rats were force-fed during 9 months and trea ted with insulin (in order to keep the blood sugar slightly above normal) and chlorpropamide (30 mg oral daily, during 7 months and 150 mg daily the last 2 months). No changes in the severity of diabetes was observed.

The third series of experiments were performed in moderate or severe alloxan diabetic dogs. Chlorpropamide was given by mouth during 60 days, 100 to 500 mg per animal. In dogs with severe diabetes no changes in the glycemia were observed. The ones with moderate diabetes showed blood sugar fall to normal or nearly normal values and a recovery or abnormal glucose tolerance curve was seen.

These changes were transitory because the diabetic state reappeared after the treatment was discontinued.

In conclusion, oral hypoglycemic compounds seem to improve transitorily experimental diabetes in animals with moderate degree of the disease, but have no recovery action on those with severe diabetes.

The preventive treatment of rats has neither benefit nor harmful effects on the incidence of diabetes.

Diferencia en la respuesta frente a la insulina de personas normales y diabéticas. A. STEINER-SZ Y J. BOUNEFOY. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

Actividad insulínica del plasma de individuos normales y diabéticos. A. STEINER-SZ. Y F. ORREGO. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

Influencia de la hipófisis en la secreción de las paratiroides. S. TALEISNIK. *(Inst. Investigaciones Médicas "Mercedes y Martín Ferreyra", Córdoba, Argentina).*

Effect of a single dose of testosterone propionate on vaginal opening. J. H. TRAMEZZANI, R. NALLAR AND H. ENCABO. *(Inst. Biología y Medicina Experimental, Labor. Neurobiología, Obligado 2490, Argentina).*

The administration of a single dose of Testosterone propionate to mice or rats less than five days old renders them permanently sterile. When the injection is given on different days after the fifth day of life the percentage of animals sterilized decreases being nil after the 20th day of life (1, 2).

In experiments attempting to clarify the mechanism of this sterility we have noticed that injected animals showed abnormalities in the opening of their vaginas.

Eighty-two "Wistar" rats were injected subcutaneously with a single dose of 0.2 mg of Testosterone propionate, dissolved in peanut oil to make a total volume of 0.1 ml per dose. One hundred sixty seven female rats were used. Animals were grouped into nine lots, and observations were carried out daily: a) 19 female rats injected on the day of birth; b) 7 female rats injected when 24 hours old; c) 12 female rats injected when 2 days old; d) 13 female rats injected when 3 days old; e) 8 female rats injected when 4 days old; f) 12 female rats injected when 5 days old; g) 11 female rats injected when 10 days old; h) 79 female rats kept as controls; i) 6 female rats injected with 0.2 ml peanut oil.

All animals injected on the day of birth failed to open their vaginas. Some of the members of this lot were kept under observation during eleven months, and still their vaginas remained unopened. Some of these rats developed colpoceles.

In rats injected when one and two days old, the injection delayed the opening of the vagina (between the 40th and the 54th day of birth, as compared with control rats who opened their vaginas between the 36th and the 45th day of life).

When testosterone propionate was injected to rats 3, 4, 5 or 10 days old, an inverse effect was obtained: the animals showed an early opening of their vaginas (between the 8th and the 13th day of life in rats injected when 3, 4 or 5 days old).

Butenandt and Kudsuz (2) have observed a precocious opening of the vagina in rats 19 to 25 days old, treated with different androgens (3). This early opening of the vagina does not require the presence of the ovaries, since it also takes place in castrated rats (4).

The results obtained suggest a metabolic conversion of testosterone to estrogens or neutral steroids possessing some estrogenic actions, which would take place only in rats older than three days.

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Efecto del glucagon sobre el fósforo inorgánico del suero en perros normales, pancreatectomizados y aloxanizados. F. DE VENANZI, T. CARNEVALI Y F.

PEÑA. *(Inst. Medicina Experimental, Cát. Patol. Gen. y Fisiopatol., Univ. Central Venezuela, Caracas, Venezuela).*

En un trabajo anterior (1) realizado en pacientes diabéticos, se demostró que el glucagon inyectado por vía venosa produce un descenso significativo del fósforo del suero que alcanza su máximo a los treinta minutos. Clasificando a los pacientes de acuerdo con el descenso de la misma substancia que ocurre después de la administración de glucosa por vía venosa, se observa que aún en los casos en que este descenso no se presenta bajo la influencia de la dextrosa, ocurre cuando se inyecta el glucagon. Se dedujo de ello, que el descenso producido por el glucagon no depende de la secreción reaccional de insulina que tiende a producir la elevación de la glucemia. Con el objeto de establecer esta conclusión experimentalmente, se realizaron investigaciones en perros normales, pancreatectomizados y aloxanizados que mostraron que el glucagon tiene efecto depresor sobre fósforo inorgánico del suero en los animales normales y diabéticos tanto pancreatectomizados como aloxanizados. En cambio, el descenso determinado por la glucosa fué defectuoso y no significativo en los perros pancreatectomizados y aloxanizados. Esto indica que el glucagon posee una acción sobre el fósforo inorgánico del suero sugiriéndose la hipótesis que el fósforo que desaparece pueda utilizarse en la producción de glucosa-1-fosfato a partir del glucógeno. No puede descartarse sin embargo la posibilidad de que el efecto sea debido al aumento de la excreción urinaria del fósforo que ha sido comunicado en otras investigaciones (2).

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(2) STAUB, A., SPRINGS, V., STOLL, F. y ELRICK, H.: *Proc. Soc. exp. Biol.* N.Y., 1957, *94*, 57.

Effect of desoxicorticosterone on water solute excretion in chronic renal failure. M. F. VILLAMIL, N. YEYATI AND I. J. DE LA RIVA. *(Centro de Investigaciones Cardiológicas, Azcuénaga 985, Buenos Aires, Argentina).*

Because patients with chronic renal failure (CRF) reabsorb a low fraction of filtered sodium it was thought of interest to investigate in them the renal response to desoxycorticosterone (DC). For this purpose a group of 17 patients with chronic renal failure was studied during maximal water diuresis under basal conditions and after intravenous or intramuscular administration of DC. Urine volume and osmolarity, C_{IN}, C_{PAH}, C_{OSM}, C_{H2O} and Na, Cl and K excretion was measured in every case. C_{PHA} and C_{IN} underwent little change except in a case in which values were doubled. Cl and Na excretion either fell, did not change or increased. Changes in urine volume were parallel to changes in Na and Cl excretion which suggested a proximal effect, distal water reabsorption being inhibited by previous water loading.

Osmolarity did not change in some cases and decreased in others suggesting a distal effect. Conversely ,in some "acute" experiments characterized by great falls in urine volume, urine became hypertonic in spite of previous water loading. This was interpreted as a consequence of the reduction in distal sodium load.

In conclusion, effect of DC in CRF was irregular, inconstant and sometimes paradoxical being apparently localized in proximal and/or distal tubules according to cases.

Actividad de distintos noresteroides progestacionales sobre el endometrio de la mujer. A. C. ZAPATA, E. BAGNATI y E. MARTÍNEZ-MONTES. *(Centro Endocrinol., Min. Asist. Soc. y Sal. Púb., Godoy Cruz 1221, Buenos Aires, rgentina).*

Se ha administrado a 133 pacientes los siguientes gestágenos: 17-alfa-vinil, 19 nortestosterona, 17-metil, acetato de alfa-etinil y 17-alfa-alil.

Las enfermas consultaron por ciclos anovulatorios, tensión premenstrual, esterilidad.

Un grupo de climatéricas, adecuadamente preparadas con estrógenos fué también sometido a esta terapéutica. Fueron controladas por temperatura basal, extendidos vaginales, moco cervical y biopsia de endometrio, realizados en forma seriadas con una micro bureta.

17-alfa-vinil: Con dosis tan bajas como 10 a 15 mg es posible obtener un endometrio en comienzo de fase secretora, cambio que se hace más evidente cuando se usan dosis de 20 mg durante 4 días.

Se ha podido obtener una secreción más o menos avanzada en el 60 % de los casos investigados cuando la dosis se elevó a 10 mg durante 10 días. En cambio cuando este compuesto sintético se comenzó a administrar en los primeros días del ciclo durante 20-22 días la mucosa fué generalmente de tipo proliferativo.

17-metil: Sólo usando 5 mg durante 3-5 días es posible observar cambios secretores iniciales. Si la dosis se aumenta a 10 mg durante 10 días se obtienen modificaciones progestacionales más avanzadas en un mayor porcentaje de casos (75 %). Con dosis de 20 mg no hay cambios significativos en la respuesta endometrial.

Acetato de 17-etinil: Ya con 2 mg algunas pacientes muestran glándulas endometriales con vacuolización subnuclear. Si la administración se repite por 3 a 5 días el aspecto progestacional es más uniforme y frecuente.

2 mg diarios durante 10 días son capaces de producir un endometrio secretor medianamente avanzado en la mitad de las pacientes tratadas. Elevando la dosis a 4 mg durante el mismo lapso se alcanza una mucosa progestacional en aproximadamente el 65 %.

17-alfa-alil: Con 5 a 10 mg y durante 4-10 días la mucosa uterina sólo presenta aspecto progestacional en el 35 % de las mujeres tratadas.

Acción de la progesterona endovenosa sobre el endometrio en la mujer normal y con alteraciones del ciclo. A. C. Zapata, E. Bagnati y E. Martínez-Montes. *(Centro Endocrinol., Min. Asist. Soc. y Sal. Púb., Godoy Cruz 1221, Buenos Aires, Argentina).*

Se efectuó la estimulación del endometrio con progesterona endovenosa a distintas dosis, en mujeres que consultaron por ciclos monofásicos y trastornos climatéricos. Las primeras fueron debidamente controladas durante algunos meses y las climatéricas con déficit hormonal tratadas previamente en forma adecuada con estrógenos. Las dosis utilizadas fueron: 20, 40, 60 y 120 mg en una sola dosis o en dos días sucesivos.

Efectuada la medicación, se realizaron biopsias inicial y seriadas cada 24 horas hasta el momento de la menstruación. A fin de disminuir las variaciones individuales en cuanto a la respuesta del endometrio, se estimuló en varios ciclos una misma paciente a distintas dosis.

Algo más de la mitad de los casos de la serie total respondieron al estímulo progestacional. Con dosis de 20 a 80 mg se obtuvo secreción completa en algunos de ellos. Sin embargo, en la mayor parte con igual dosis o algo superiores, el endometrio mostró sólo secreción incompleta. No se consiguió maduración secretora, evidenciando ausencia de correlación entre dosis y actividad, en algo menos de la mitad de las observaciones.

Debe destacarse que la forma de la respuesta del endometrio fué en muchos casos de acuerdo a la particularidad de las enfermas. Algunas respondieron en forma regular a las distintas dosis con endometrio secretor, en otras, fué variable y en un tercer grupo, la mucosa del endometrio no modificó su tipo proliferativo.

La disgregación de la mucosa uterina se produjo en el 80 % de las pacientes entre 1 y 4 días luego de interrumpida la medicación.

Los controles posteriores de los ciclos anovulatorios mostraron ausencia de nidificación con la terapéutica.

A las dosis utilizadas, no se observaron efectos colaterales indeseables.

Effects of oxytocin on diabetes insipidus. G. Zeballos, H. Croxatto and L. Cardemil. *(Lab. Fisiología, Facultad de Filosofía y Educación, Universidad de Chile, Santiago, Chile).*

The effects of the subcutaneous injection of 5 mU of oxytocin (Syntocinon Sandoz), of 2.5 mU of l-vasopressin and of both combined were studied on normal rats as well as on rats showing diabetes insipidus due to an injury of the infundibulum (15 to 50 ml of urine every 8 hours). The controls were injected with a 9 ‰ solution of NaCl. Immediately after the injection the rats were overloaded by means of a catheter introduced into the stomach (5 % of b/w). The diuresis was studied during 3 hours.

Oxytocin elicited its usual response in normal rats by stimulating the excretion of sodium and potassium whereas vasopressin produced antidiuretic effects. Simultaneous injec-

tion of both hormones, oxytocin-vasopressin, resulted in effects which were half way between those obtained with oxytocin and vasopressin injected separately.

In rats with diabetes insipidus, oxytocin reproduces the effects obtained in normal animals particularly regarding the excretion of sodium, the elimination of which increased strikingly. Its effects on diuresis were rather scanty. The antidiuretic effect of vasopressin was more intense in these than in the normal animals. The injection of both hormones oxytocin-vasopressin on lesioned rats resulted in a greater excretion of sodium and a lower diuresis than those obtained in normal rats.

The results obtained with rats having diabetes insipidus would show that oxytocin facilitates the output of water and sodium independently from the activity of vasopressin.

ENFERMEDAD DE CHAGAS
FISIOPATOLOGÍA
CHAGAS DISEASE
PHYSIOPATHOLOGY

Biosíntese dos ácidos nuclêicos em *Trypanosoma cruzi* em relação às perpectivas para a quimioterapia do mal de Chagas. J. F. Fernandes e O. Y. Castellani. *(Depto. Histologia y Embriologia, Fac. Medicina São Paulo, Univ. São Paulo, Brasil).*

A exigência de meios de cultura extremamente ricos para se cultivar o *T. cruzi* é certamente devida à pequena habilidade biossintética do parasita. Foi possível mostrar que o protozoário é incapaz de sintetizar eficientemente as purinas e pirimidinas de que necessita para a sua multiplicação, fato que não ocorre na maioria da população celular, inclusive nas células parasitadas pelo *T. cruzi*. Esta observação serviu para sugerir o emprêgo de análogos de purinas (especialmente o aminonucleosídio da estilomicina) e pirimidinas, como inibidor da biossíntese de ácidos nuclêicos, estudada nas formas de cultura do parasita, no seu ciclo biológico em cultivo de tecidos, bem como em infecção experimental de camondongos.

Hipersensibilidade bronquiolar à acetilbetametilcolina na moléstia de Chagas. R. A. Godoy. *(Depto. Clínica Médica, Fac. Medicina Ribeirão Prêto, Univ. São Paulo, Ribeirão Prêto, SP, Brasil).*

Pneumogramas foram obtidos em indivíduos normais e em portadores de moléstia de Chagas, antes e durante a ação de pequenas doses de cloreto de acetilbetametilcolina (0.05 a 0.1 mg/kg de pêso corporal), injetadas por via intramuscular.

A análise dos gráficos mostrou que em alguns pacientes chagásicos, ao lado de hiperpnéia, havia deslocamento geral do traçado no sentido inspiratório e encurtamento da fase expiratória, o que pode ser explicado por constricção bronquiolar. Em apôio dessa explicação vem o fato de que dois pacientes que tiveram êsse tipo de resposta respiratória exibiram dispnéia e sibilos durante a ação da droga.

Com os contrôles normais não tiveram alteração significativa da forma dos pneumogramas, concluiu-se que pacientes chagásicos podem ter musculatura bronquiolar hipersensível à ação do agente colinérgico empregado.

Tempo de trânsito esofágico em portadores de moléstia de Chagas. R. A. Godoy e N. Haddad. *(Deptos. de Clínica Médica e Higiene e Medicina Preventiva, Fac. Medicina Ribeirão Prêto, Univ. São Paulo, Ribeirão Prêto, SP, Brasil).*

À fluoroscopia, foi determinado o tempo gasto por uma pequena quantidade (15 a 20 ml) de uma suspensão espêssa de sulfato de bário, entre a sua passagem pelo nível do manúbrio esternal até a sua entrada no estômago.

Num grupo de indivíduos tomados como contrôle, o tempo de trânsito assim determinado foi de 6.2 ± 3.2 seg.

Por êsse método foram estudados indivíduos portadores de moléstia de Chagas, em que o diagnóstico foi feito ùnicamente na base da positividade da reação de Machado-Guerreiro. Dêsse grupo, apenas um paciente se queixava de discreta disfagia e o exame

radiológico convencional não revelou aumento de calibre do órgão.

O estudo pela técnica citada mostrou que quase 20 % dêsses pacientes tiveram tempos de trânsito superiores a 9.4 seg.

Podemos concluir que um número significativo de pacientes chagásicos, sem sintomas relacionados ao esôfago e sem dilatação do órgão ao exame radiológico convencional, mostra evidência de lesão funcional do órgão, revelada pelo aumento do tempo de trânsito determinado por técnica adequada.

Estudio del tiempo de tránsito esofágico en enfermos chagásicos. C. Iñiguez-Montenegro y H. Dalvit. *(Fac. Medicina, Córdoba, Argentina).*

Quantitative studies of neuronal destruction in Chagas disease. F. Köberle. *(Depto. de Patología, Fac. Medicina, Ribeirão Prêto, USP, Brasil).*

According to our new conception of Chagas disease two different pathological processus should be distinguished:

1) Chagas disease, in the strict sense, that is, infection with *Trypanosoma cruzi*, with an acute septicaemic phase, which after the development of specific resistance, passes into the chronic phase with few parasites.

2) Sequelae of Chagas disease or late manifestations which develop as a result of extensive destruction of ganglion cells in the peripheral autonomic and/or the central nervous system during the acute phase.

Based on this conception, quantitative studies have been carried out of the number of ganglion cells in human beings and animals comparing normal with chagasic cases. These researches were performed in the hollow muscular organs (Oesophagus, colon and bronchi), in the heart and in different levels of the spinal cord. In the chagasic cases, a remarkable diminution of ganglion cells in the central and peripheral nervous system was verified, especially in chagasic cases with late manifestations as cardiomegaly, megaoesophagus, megacolon and bronchiectasy.

Based on these quantitative studies, it has been considered, that destruction of ganglion cells in certain territories of central and/or peripheric nervous system is essentially responsible for late symptomatology and morphologic manifestations in the chronic phase of Chagas disease.

The autonomic heart rate regulation of rats with chronic Chagas disease. E. M. Krieger and F. G. Alcantara. *(Deptos. Fisiología e Patología, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

In two groups of 6 and 8 rats which subsisted the acute form of the disease determined by the inoculation of the *Trypanosoma cruzi* the autonomic regulation of the heart rate was studied. During the acute phase the animals presented the typical manifestation of the disease and the trypanosomes were found in the blood. At the moment of the present study the animals were in good clinical condition. After pentobarbital anesthesia the heart rate and the heart rhythm (ECG) were similar in the control and in the inoculated animals.

The electrical stimulation (Grass stimulator) of the cervical sympathetic system and the administration of epinephrine were used to test the sympathetic effects on the heart rate. Epinephrine was injected after atropinization at normal body temperature and during hypothermia (until approximately 20°C). The heart rate responses of the rats with chronic Chagas's disease were no different from those presented by the control animals.

When the vagus nerve was stimulated, the threshold voltages (40/sec - 10 sec total duration) for the bradycardic responses production were almost the same in the control and in the inoculated animals. However, the bradycardia determined by supramaximal stimuli (with less than 30 sec total duration) was greater in the rats with Chagas's disease. The bradycardia produced by the injection of acetylcholine and mecholyl chloride was also more intense in the inoculated rats, although the duration of the effects was almost the same.

Reacciones indirectas para el diagnóstico de la enfermedad de Chagas aguda. J. L. Minoprio, A. Durandeau, R. De Vecchi y E. Lemos. *(Cát. Enfer-*

medades Infecciosas, Fac. Medicina, Univ. de Cuyo, Argentina).

Los autores han investigado durante varios años los trastornos de las proteínas plasmáticas durante la forma aguda de la enfermedad de Chagas. Sugieren la reacción de Wunderly-Wuhrmann, empleada en la investigación de la disproteinemia ocasionada por el Kala-Azar, para el estudio de los disturbios proteicos determinados por la enfermedad de Chagas.

Esta reacción permite constatar variaciones mínimas de la disproteinemia y, dada su simplicidad y constancia, se presta al diagnóstico de las formas agudas de la enfermedad, en casos clínicamente inaparentes, en medios endémicos donde la reacción de Machado-Guerreiro muestra un elevado índice de positividad. Puede también mostrar el pasaje a las formas crónicas de la enfermedad, junto con la investigación de la positividad parasitológica.

Llaman también la atención para el hecho de que otros procesos agudos, y entre estos en especial la brucelosis, dan reacción de W-W positiva.

En sus comprobaciones obtuvieron resultados análogos a los ya descriptos por otros autores, esto es: disminución de las seroalbúminas (acentuada en los edematosos) asociada a un aumento de las alfa2-globulinas, discreta disminución de las beta-globulinas y moderado aumento de las gama-globulinas.

Curvas glicêmicas anormais em portadores da forma crônica da moléstia de Chagas. L. C. F. Reis, H. L. Oliveira e C. B. Vieira. *(Depto. Clínica Médica, Fac. Medicina, Univ. São Paulo, Ribeirão Prêto, SP, Brasil)* .

Foram estudadas curvas glicêmicas obtidas após administração oral de glicose, em pacientes portadores da forma crônica da moléstia de Chagas. Observou-se hiperglicemia provocada anormalmente alta, com acme precoce iniciando-se a fase descendente da curva em tempo normal e atingindo-se os níveis glicêmicos de jejum com certo atraso.

As curvas glicêmicas ora em investigação, obtidas após injeções de adrenalina ou de glicagônio, não se mostraram até o presente diferentes das dos contrôles normais.

Os dados apresentados são preliminares e ainda não permiten seguras interpretações. Como, porén, já estão bem demonstradas lesões disseminadas em neurônios, particularmente em grandes áreas de invervação autônoma visceral, na moléstia de Chagas, cabe aquí, como hipótese a considerar na interpretação fisiopatológica da anormalidade da curva, a possibilidade de uma perturbação no mecanismo reflexo responsável pela regulação dos niveis da glicemia.

Changes in the size of hypertrophic parotid glands and variation of serum amylase in patients with Chagas disease and megaesophagus treated by sub-total esophagectomy. C. Bühler Vieira, L. H. Câmara-Lopes and W. Menegucci. *(Depto. Clínica Médica, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

Following sub-total esophagectomy (first step of the complete procedure), the patients being fed through stomach tube, a marked decrease in the size of previously hypertrophic parotid glands was observed in 6 cases. In those patients showing hyperamylasemia before the operation, a fall of serum amylase was also observed.

After the second operation, when a cervical esophagogastric anastomosis is performed, the patients started feeding themselves by mouth again. Shortly thereafter, the parotid glands increased in size and the serum amylase rose somewhat; the glands however, never reached the size observed before the first operation.

In two patients the complete procedure (sub-total esophagectomy and cervical gastro-esophageal anastomosis) was performed in one operation. A decrease of previously enlarged parotid glands was also noticed, but less markedly than in those undergoing esophagectomy and fed by stomach tube. Only such moderate change was also observed in two other patients treated respectively by partial infra-aortic esophagectomy and Heller's operation.

The hypertrophy of salivary glands in patients with megaesophagus is frequently explained as a result of abnormal reflex estimulation arising from dilated esophagus. The above observations are not consistent with such interpretation. The salivary glands seem to be hypersensitive to other reflex stimuli, so that

their hypertrophy persists and may even develop in the absence of esophagus.

Esophageal motor response to cholinergic agents in chronic Chagas disease. C. BÜHLER VIEIRA AND R. ALVES DE GODOY. *(Depto. Clínica Médica, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

The motor activity of the esophagus was studied by kymographic method in 40 patients with chronic Chagas disease. None of them had dysphagia; in every one the esophagus was roentgenologically normal when examined by conventional techniques. Megacolon was demonstrated in 17 of them.

The results obtained in such group are compared with those given by 6 patients with Chagas disease and overt megaesophagus and by 10 normal controls. As cholinergic agents, metacholine and pilocarpine were used, intramuscularly injected as 1 % solution; individual doses were calculated for the body weight (either 0.05 or 0.10 mg per Kg).

In the cases of megaesophagus, as previously reported, a hyperreactive response was found. A similar reaction, only lesser in degree, was recorded in half of the patients with Chagas disease and seemingly normal esophagus; these hyperreactors were 1/3 of the patients with no demonstrable organic change in the digestive tract and 4/5 of those showing megacolon.

Testing the reactivity to cholinergic drugs, therefore, seems to be a reliable means to diagnose asymptomatic aperistalsis of esophagus in Chagas disease. The results were particularly clear when metacholine (0.05 mg per Kg) was used.

FARMACOLOGÍA
PHARMACOLOGY

Inibição da liberação de histamina do trem posterior do rato pelo aquecimento "in vivo" a 45° C. A. ANTONIO. *(Depto. Farmacologia, Fac. Medicina Ribeirão Prêto, São Paulo, Brasil).*

Em trabalhos anteriores (1, 2), foi mencionado o efeito do aquecimento prévio (45°C durante 25 a 30 minutos) do trem posterior do rato intacto, na ulterior liberação de histamina pelo 48/80. A diminuição considerável das quantidades de histamina liberadas após o tratamento térmico é uma indicação de que o mecanismo de liberação pode ser afetado *in vivo*, da maneira como foi demostrado *in vivo* com fragmentos de pulmão de cobaia ou de mesentério de rato.

No trabalho que se segue o fenômeno foi confirmado em maior número de animais e feita a verificação de que o teor de histamina não é alterado significativamente quando comparados os teores totais em fragmentos de pele retirados de áreas da pele mantidas a 37°C e áreas simétricas submetidas ao aquecimento a 45°C durante 30 minutos.

A demonstração de que o tratamento térmico aplicado ao rato intacto afeta o mecanismo de liberação da histamina, no mesmo sentido e com igual intensidade que nas experiências *in vitro* descritas na literatura, é uma indicação de que não se trata de um artefato nestas últimas experiências (*in vitro*) e que mesmo em condições normais de oxigenação e meio interno, algum metabolito essencial para o processo é eliminado pelo aquecimento a 45°C durante 25 a 30 minutos.

(1) ANTONIO, A. e ROCHA e SILVA, M.: *Efeito de drogas na produção do "edema térmico* 44-45°C *na pata do rato. — XII Reunião Anual S. B. P. C., julho,* 1960. *Ciência e Cultura (em publicação).*

(2) ROCHA e SILVA, M. e ANTONIO, A.: *Release of Bradykinin and the Mechanism of Production of a "Thermic Edema* (45°C)" *in the Rat's paw. Med. Exp.,* 3: 371-3882 (1960).

Mechanism of action of purified fractions from *Ascaris lumbricoides* on isolated guinea pig ileum. W. T. BERALDO, W. DIAS DA SILVA AND J. PUDLES. *(Deptos. Fisiologia e Bioquímica, Fac. Medicina, UMG., Belo Horizonte, Brasil).*

Gazzinelli *et al.* (1961) obtained from *Ascaris lumbricoides* a purified fraction which caused contraction of the guinea pig ileum. Chemical analysis showed to be a complex substance containing sugars and protein. The present communication deals with the mechanism of contraction induced by purified *As-*

caris fraction, on the guinea pig ileum. The addition of 5 to 20 μg of a purified *Ascaris* fraction to the perfusing bath containing the guinea pig ileum induced a strong contraction. A second addition of the same dose of *Ascaris* fraction was either ineffective or caused a smaller effect, followed by a refractory state on further addition of the fraction (desensitization). The contraction was observed in about 85 per cent of the ilea. Previous heating of the ileum at 45°C inhibited the response to *Ascaris* fraction, however, the ileum still reacted to anaphylatoxin. These experiments exclude the participation of the latter and suggest an antigen-antibody reaction as the mechanism of this contraction. Previously desensitized guinea pig ileum could be again resensitized by incubation at 35°C, for 2 hours, with spleen extract or spleen protein obtained by adding ammonium sulphate to 50 % saturation. Spleen extracts obtained from guinea pigs whose ileum did not respond to *Ascaris* fraction also were unable to transfer the sensitivity. On the basis of these findings, it is suggested that the action of purified *Ascaris* fraction on isolated guinea pig ileum is due to an antigen-antibody reaction.

GAZZINELLI, G., GUIA, M. M., NEVES, A. G. A., PUDLES, J., BERALDO, W. T. AND DIAS DA SILVA, W.: *Nature*, (Lond.), 1961, *190*, 813.

Effect of intracerebral injection of compound 48/80 in the rat. R. J. CARMO AND W. DIAS DA SILVA. *(Depto. Fisiologia, Fac. Medicina, UMG., Belo Horizonte, Brasil).*

Effects of intracerebral injections of compound 48/80 in the mouse were described by Rocha e Silva (1949). In this paper, the effects of intracerebral injections of this drug in the rat, were studied using Halley's technique (1957) adapted for this animal. The volume of injected solutions did not exceed 0.1 ml. Tyrode solution and saline were injected in control animals. With a dose of 60 μg/100g of 48/80, espasticity and hypersensitivity to touch was observed 30 to 180 seconds after injection. A few animals showed generalized convulsions and died. Cyanosis, oedema and pruritus were sometimes observed. With 15 μg, similar but lighter symptoms appeared. The injection of 10 μg produced spasticity and incoordination of movements followed by "opisthotonus"; there was no reaction to sound and the threshold to touch seemed to be higher. The animal usually recovered in about 40 minutes. When 5 μg were injected the animal fall to one side and moved in circle. One μg was just sufficient to produce light neurologic symptoms. From the above described results it seems clear that the intensity of symptoms is related to the dosage. The "delirium ambulatorium", described by Rocha e Silva (1949) after injection of 48/80 in the mouse, was not observed by us. One daily intracerebral injection (10 μg/100g) of compound 48/80 induced tachyphylaxis, that is, the animal did not respond to further injections of the drug. Depletion of histamine was not observed in various regions of C. N. S. after this procedure. Previous injection of antihistaminics (Benadryl and Pyrabenzamine) did not alter the symptoms caused by compound 48/80. Serotonin (200 μg/100g) produced different neurologic symptoms. Serpasol (25 μg/100g) produced neurological symptoms similar to 48/80 plus strong pruritus. Repeated injections of Serpasol induced desensitization to this drug, however, the animals still responded, when 48/80 was injected, showing the usual symptoms. On the basis of these findings it is suggested that histamine and serotonin are not involved in the neurologic symptoms induced by intracerebral injection of compound 48/80 in the rat.

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HALEY, T. J. AND MCCORMICK, W. G.: *Brit. J. Pharmacol.*, 1959 *12*, 12.

Estudo electrofisiológico da ação convulsivante central da morfina, codeína e tebaína *. A. P. CORRADO E V. G. LONGO. *(Depto. Farmacologia, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil e Laboratorio Chim. Terap. Istituto Superiore di Sanità, Roma, Italia).*

As acentuadas afinidades químicas dos três alcalóides naturais do ópio, morfina, codeína e tebaína, têm dado motivo a intensivos estudos sôbre a relação entre estrutura química e ação farmacológica. Êstes estudos têm-se limitado às diferenças qualitativas e quantitati-

vas dos efeitos obtidos pela substituição de uma ou ambas oxidrilas do núcleo fenantrênico da morfina. A literatura é pobre, entretanto, em dados referentes à análise dos mecanismos neurofarmacológicos que regulam e acompanham a passagem da característica depressão provocada pela morfina à sintomatologia francamente convulsivante observada com a tebaína.

Na presente investigação reexaminamos êse problema usando técnicas electrofisiológicas, procurando relacionar os fenômenos neurofisiológicos com os sinais observados no decorrer da intoxicação aguda provocada por êsses alcalóides. Em resumo estudamos:

1. A variação da atividade elétrica registrada em diferentes níveis do eixo cérebrospinal em coelhos anestesiados, curarizados e mantidos em caixa de contenção para o registro simultâneo da pressão arterial femoral e do traçado eletroencefalográfico (EEG). Os elétrodos nas estruturas subcorticais foram implantados segundo a técnica de Monnier (1).

2. O efeito da aplicação local dos alcalóides, no eletrocorticograma (EEG) de coelhos não anestesiados.

3. Influência na inibição espinal ou direta em gatos espinalizados e sob leve anestesia cloralósica (20 a 30 mg/kg e.v.).

Com base nos nossos resultados parece existir uma correlação entre as diferenças notadas na sintomatologia da intoxicação aguda e as modificações da atividade elétrica cerebral. O quadro elétrico provocado pela morfina que causa o aparecimiento de convulsões preponderantemente clônicas-caracteriza-se por alterações corticodiencefálicas, enquanto que a tebaína-cujas convulsões são preponderantemente tônicas-induz o aparecimiento de um paroxismo de ondas de 20-25 c/seg. ao nível espinocerebelar. Com a codeína, cujas convulsões são do tipo tônico-clônicas observa-se o aparecimiento de um quadro EEG com característicos intermediários aos precedentes.

Quando aplicados no córtex cerebral, na mesma concentração (até 0.2 %), os 3 alcalóides apresentam o mesmo gráu de atividade pois determinam o aparecimiento de espículas (spikes) primeiramente localizadas e que em seguida se generalizam para as demais derivações.

A tebaína, em dose de 1 a 2 mg/kg e.v., bloqueia a inibição espinal primária, ao passo que a morfina e a codeína, mesmo em doses superiores a 10 mg/kg e.v., não causam nenhum efeito sôbre essa inibição mas, pelo contrário, provocam uma diminuição do reflexo monossináptico.

Tais efeitos da tebaína na medula espinal, no EEG e ECG, vêm demonstrar as notáveis semelhanças entre êsse alcalóide e a estricnina.

(1) MONNIER, M. E LAUE, H.: *Helv. physiol. Acta*, 1953, *11*, 73.

* (Trabalho realizado no "Laboratorio di Chimica Terapeutica" do Istituto Superiore di Sanità, Roma, Italia.)

Pharmacological characteristics of anephrotensin obtained from the frog *Caliptocephalella gayi*. H. CROXATTO, E. LABARCA AND S. AGUIRRE. *(Labor. Fisiología, Fac. de Filosofía y Educación, Univ. de Chile, Santiago, Chile).*

From the serum of the frog *Caliptocephalella gayi* submitted to the same procedure used for obtaining anephrotensin from the serum of other species, a polypeptide showing characteristics quite similar to those displayed by angiotensin and pepsitensin, is obtained. It is destroyed by the different proteolytic enzymes. It increases the arterial blood pressure of either normal or nephrectomized rats, cats and dogs. It elicits a contraction of the duodenum and uterus of the rat. However, on the basis of equivalent pressor doses, the preparations obtained are 15 to 20 times less active than angiotensin when assayed on isolated duodenum and uterus.

When the kidneys are excluded one hour before bleeding the results are similar to those obtained in the control animals having kidneys. Due to its hypertensive effects on nephrectomized rats it can be reckoned that 1 ml of serum yields an amount of this polypeptide which is equal to 0.10 μg of angiotensin-valyl 5. The formation of this polypeptide will be discussed: a) in relation to the findings of other authors which have established the absence of renin in the kidney of amphibians, and b) in relation to the possibility of inducing renoprival hypertension in these animals.

Histamine release and mast cell damage induced by purified fractions

from *Ascaris lumbricoides*, "in vitro". Effect of inhibitors of anaphylaxis. W. DIAS DA SILVA, W. T. BERALDO AND J. PUDLES. *(Deptos. Fisiologia & Bioquimica, Fac. Medicina, UMG., Belo Horizonte, Brasil).*

It was demonstrated by Gazzinelli *et al.* (1961), that purified fractions from *Ascaris lumbricoides* induced an anaphylactic-like shock in the guinea-pig. It was also showed that *Ascaris* fractions caused contraction of isolated guinea-pig ileum followed by desensitization. Recently, Beraldo, Dias da Silva and Pudles (1961), on basis of *in vivo* and *in vitro* experiments suggested that an antigen-antibody reaction was involved in the mechanism of action of the *Ascaris* extract on guinea-pig In this paper results of *in vitro* experiments were reported in which guinea pig tissues (lung and mesentery) were incubated with purified *Ascaris* fractions. It was found that *Ascaris* fractions released about 10 per cent of total amount of tissue histamine. Histological examination of mesentery showed a reduction of 50-70 per cent in the number of mast cells. Mast cells damage very similar to that found in anaphylaxis was observed. The release of histamine and mast cells alterations were observed only in those guinea pigs which ilea contracted when in contact with *Ascaris* fractions. The mast cell alterations occur simultaneously with histamine release and both processes are inhibited by iodoacetate, phenol, calcium lack and previous heating of the tissue to 45°C. These results show that *Ascaris* fractions act on guinea pig tissue by a mechanism very similar if not identical to anaphylaxis.

BERALDO, W. T., DIAS DA SILVA, W. AND PUDLES, J.: *Brit. J. Pharmacol.*, 1961, (in press).

GAZZINELLI, G., GUIA, M. M., NEVES, A. G. A., PUDLES, J., BERALDO, W T. AND DIAS DA SILVA, W.: *Nature*, 1961, *190*, 813.

Enzymatic inactivation of substance "V" and bradykinin. J. C. FASCIOLO, K. HALVORSEN BINIA AND O. CARRETERO. *(Depto. Fisiologia, Fac. Medicina, Univ. de Cuyo, Mendoza, Argentina).*

The enzymatic inactivation of substance "V", and bradykinin, by blood plasma and different tissues, was studied. The kidney was found to be the most active tissue, followed by the spleen, liver, brain, red cells and blood plasma.

The optimum pH for the renal enzyme was between 7 and 10, having slight activity below 6. The optimum temperature was found to be between 35°C and 50°C, the enzyme being destroyed above 55°C.

By acidifying the renal extract at pH 3.5, 30 minutes at 37°C, its capacity to destroy *Bothrops* bradykinin is lost, while this effect on substance "V", is partially preserved. Blood plasma, similarly treated at pH 4.5, loses its power to destroy *Bothrops* bradykinin, but keeps its effects on substance "V".

Bothrops bradykinin appears not to be destroyed by acidified plasma, which, on the other hand, inactivates substance "V", highly purified trypsin-bradykinin, and the nonapeptide synthesized by Boisonnas et al., which seems to be identical with the latter.

The differences found could be due to structural differences, or to the presence of contaminants in the less purified *Bothrops* bradykinin preparation.

Effect of partially purified fractions from peptone on the guinea pig ileum. L. FREIRE-MAIA AND G. A. NEVES. *(Deptos. Fisiologia e Bioquimica, Fac. Medicina, UMG., Belo Horizonte, Brasil).*

Using paper and zone starch electrophoresis and ammonium sulphate, alcohol and acetone precipitations, we were able to confirm experiments reported by Beraldo and Zanotto (1960) concerned to the presence in peptone of an active substance which causes contraction of the isolated guinea pig ileum. In those experiments in which zone starch electrophoresis was used, a quite active material was obtained, 0.5 to 2 μg added to a 10 ml perfusing bath causing contraction; 2 μg of this fraction produced a contraction equivalent to 0.03 μg acetylcholine. The spasmogenic effect of this fraction was not influenced by antihistaminics; on the other hand, it was blocked by atropine and potentiated by eserine. Although starch electrophoresis gave us a quite active material, the separation of active fractions was not so good, the activity spred out among the several fractions. So, it was de-

cided to try the purification of the active substance of peptone by precipitation with acetone. The results of these experiments showed that the supernatant caused contraction of the ileum, whereas the precipitate decreased its tonus. The pharmacological properties of the supernatant showed very similar to, if not identical, with those fractions obtained by electroforesis. Furthermore, the activity of the supernatant disappeared after incubation with chymotrypsin. The results of these experiments suggest the presence in the peptone of an active substance or substances, probably of a peptide nature, which contracts the guinea pig ileum through a cholinergic mechanism of action.

BERALDO, W. T. AND ZANOTTO, A.: *Brit. J. Pharmacol.*, 1960, *15*, 224.

Dose-effect relationship of morphine, evaluated by the algimetric method of electric stimulation of rabbit tooth pulp. S. GUERRERO, C. PAEILE AND S. LECANNELIER. *(Inst. Farmacología, Universidad Chile, Santiago, Chile).*

The dose-effect relationship of morphine analgesia was evaluated with the algimetric method of electric stimulation of the rabbit tooth pulp. Rectangular electric stimuli of 5 msec with a frequency of 5 per sec and variable voltage were used. Threshold voltage was estimated by the characteristic masticatory response. Three doses of morphine HCl, 2.5, 5 and 10 mg/kg, i.v. were tested. An increase of the threshold over the basic one was considered analgesic effect and it was statistically analysed in two different ways: 1) by relating the maximum increase of threshold, expressed in per cent of the basic, with the logarithm of the dose and 2) by studying the relationship of the square root of the area formed by the increase of the threshold, in per cent of basic, and the time in hours, with the logarithm of the dose. Both methods of analysis showed that the effect increases linearly with the logarithm of the dose and that significant results could be obtained with a rather small number of experiments.

Effect of adrenergic-blocking drugs on morphine analgesia in rabbits. S. GUERRERO, C. PAEILE AND C. MUÑOZ. *(Inst. Farmacología, Univ. Chile, Santiago, Chile).*

It has been previously reported that the EEG changes induced by morphine in conscious unrestrained rabbits are markedly reversed with dichloroisoproterenol (DCI) (Goldstein L. and Muñoz, C.: Fed. Proc., 1961, *20*, 319). It was interesting to study whether DCI and other adrenergic-blocking drugs could also modify morphine analgesia. The algesiometric test of electric stimulation of rabbit tooth pulp was used; DCI, phenoxybenzamine (PB) and phentolamine (P) were tested on their influence upon pain threshold as well as on their effect on the pain threshold changes induced by morphine. DCI (4-8 mg/kg) neither changed the threshold level nor modified the effect of morphine. PB (0.5 2 and 4 mg/kg) significantly increased the pain threshold and potentiated the analgesic effect of morphine. P (5-10 mg/kg) did not alter the threshold level, but in doses of 10 mg/kg it decreased significantly the analgesic effect of morphine.

Efecto del diclorhidrato de 7-metoxi-3-cloro-9- (1-metil-4-dietilamino)-butilamino-acridina en placa mioneural, ganglio simpático cervical superior, áreas corticales y núcleos subcorticales. R. GUERRERO-FIGUEROA. *(Fac. Medicina, Univ. Nac. Colombia, Bogotá, Colombia).*

El objeto de este trabajo es aclarar a qué nivel actúa el clorhidrato de quinacrina y cuál es su mecanismo bioquímico. El plan de estudio fué realizado en preparación neuromuscular de ratón, ganglio simpático cervical superior, áreas corticales y núcleos subcorticales del gato.

La administración del clorhidrato de quinacrina en preparación neuromuscular de ratón, aumenta el umbral, disminuye la amplitud de la respuesta al estímulo único indirecto; igual efecto realiza en el tétano imperfecto y perfecto. Desaparece el efecto del clorhidrato de quinacrina cuando se administra metilsulfato de Neostigmine. No hay modificaciones al estímulo directo muscular. En ganglio simpático cervical superior no origina cambios facilitatorios e inhibitorios, ni modificaciones del umbral a estímulos únicos y repetitivos.

Las post-descargadas evocadas por la estimulación eléctrica de áreas corticales y subcorticales fueron controladas en relación al umbral o voltaje, amplitud, difusión y duración total de las post-descargas. La administración del clorhidrato de quinacrina en este grupo de animales está caracterizada por cambios en aumento de umbral o voltaje, disminución de la amplitud, acortamiento de la difusión y del tiempo total de las post-descargas.

La actividad asincrónica la convierte en sincrónica. Postulamos que el clorhidrato de quinacrina actúa por un mecanismo de inhibición o destrucción de la acetilcolina. Esta droga no tiene efecto en las sinapsis cuyo mediador químico son las catecolaminas.

Some peculiarities induced by nalorphine in the white mice implanted with pellets of morphine. F. HUIDOBRO AND C. MAGGIOLO. *(Depto. Farmacología, Univ. Católica, Santiago, Chile).*

When nalorphine was injected to mice absorbing an important daily quantity of morphine, an intense abstinence syndrome is induced. The administration of nalorphine day by day induced an attenuation of the syndrome intensity.

In order to study better the phenomenon, two groups of mice were employed. The first one received the same dose of nalorphine, injected 3 times a day; in the other, nalorphine was administered in increasing amounts. The already indicated results were confirmed. Furthermore, it was possible to observe as the animals received more and more injections of nalorphine, symptoms of morphinism which increased in such a way that some mice died after some days. When nalorphine was discontinued, morphinic symptomatology decreased gradually.

If levallorphan was employed instead of nalorphine, the same results above described were observed.

If nalorphine was administered as a pellet, the abstinence syndrome symptomatology was intense and prolonged for 24-28 hours.

Norepinefrina y epinefrina en orina, cerebro, suprarrenales y bazo de ratones normales o aislados y tratados con imipramina. J. A. IZQUIERDO Y A. M. BISCARDI. *(Cát. Farmacología Experimental, Fac. Medicina y Bioquímica, Buenos Aires, Argentina)*

Se determina diariamente por fluorometría, durante 77 días, la nerepinefrina (NE) y epinefrina (E) en orina (20 horas) de 24 ratones machos (aprox. 20 g), mantenidos en la penumbra y distribuídos en 2 lotes de 6 cada uno (normales) y aislando individualmente a los 12 restantes. Los días 53°, 63° y 70° se inyecta imipramina (I) 3 mg/kg i.p. a 6 normales y 6 aislados. La experiencia se finaliza determinando NE y E en cerebro, suprarrenales y bazo de los 24 ratones.

En los ratones con modificaciones en la conducta por aislamiento, aumenta en cerebro el contenido de NE, y particularmente de E. En orina, el aumento de NE es mayor que el de E, disminuyendo ambas catecolaminas en suprarrenales (más la NE) y en bazo.

La I en cerebro de ratones normales y aislados determina aumento escaso de NE, y disminución leve de E. en el normal; esta última aumenta en el aislado. En orina de normales y aislados aumenta la eliminación de NE y E, mientras la NE aumenta en suprarrenales, las dos catecolaminas disminuyen en el bazo de ambos grupos de animales.

Se hacen consideraciones sobre los valores de NE y E en ratones aislados y los obtenidos con fármacos que modifican la conducta animal.

Influencia de la imipramina en las acciones presoras y en el ECG de la epinefrina, serotonina y angiotensina. A. J. KAUMANN, J. COUSSIO Y J. A. IZQUIERDO. *(Cát. Farmacología Experimental, Fac. Farmacia y Bioquímica, Buenos Aires, Argentina).*

Se comprueba en 20 perros las influencias de la imipramina en las respuestas presoras y en el ECG provocadas por la epinefrina, norepinefrina, serotonina y angiotensina. En 4 perros reserpinizados 48 horas antes se comprueban los efectos de la imipramina, de norepinefrina y de epinefrina.

1. La imipramina provoca moderadas modificaciones en el cronopismo y en la repolarización.

2. La epinefrina después de la imipramina determina respuestas hiperpresoras variables y el ECG es semejante al observado antes de la imipramina.

3. La norepinefrina después de la imipramina provoca hiperpresión más intensa, prolongada taquicardia y alteraciones significativas en la repolarización cardíaca.

4. Las hiperpresiones de la serotonina y angiotensina son más acentuadas después de la imipramina, en cambio las respuestas del ECG no son influenciadas.

5. En el perro reserpinizado 48 horas antes, la imipramina anula la hipopresión provocada por aquélla, restableciéndose la normopresión. El ECG es similar a 1. La mayor hiperpresión de la norepinefrina después de la reserpina, se acentúa aún más con la imipramina. Las modificaciones de la norepinefrina en el ECG después de la imipramina son similares a 3.

6. Los efectos de la imipramina, norepinefrina, serotonina y angiotensina después de la imipramina, no son casi influenciados por un bloqueante ganglionar (azametonio).

Studies on the abstinence syndrome to morphine in mice. C. MAGGIOLO AND F. HUIDOBRO. *(Depto. Farmacología, Univ. Católica, Santiago, Chile).*

The administration of nalorphine in mice implanted with morphine produced an intense syndrome with diverse and variable symptoms; because of this, a comparative evaluation was difficult.

If the dose of morphine implanted was adequate, symptoms of abstinence appeared 12-24 hours after the morphine implantation. Afterwards, their intensity increased and reached a steady state that was maintained.

Implants of pellets which induced a small daily absorption of morphine produced a very attenuated syndrome; the physical dependence was exaggerated if larger doses of morphine were daily absorbed.

When morphine was injected, only in those cases that the drug was administered several times a day, could nalorphine induce the abstinence syndrome. Related with those observed in the cases of morphine implantation, its intensity was lower and it appeared only after some days.

Some hours after the pellet removal, an abstinence syndrome was manifested which was not observed when injections of morphine were discontinued.

The importance of the dose and the continuity in the administration of morphine to produce the abstinence syndrome was emphasized.

Determination of the increase of the oxygen consumption in the toad *(Bufo spinulosus)* induced by the action of antirrheumatic drugs. J. MARCELIN, L. BARDISA, L. FRANCKE Y J. CONCHA-BARAHONA. *(Inst. Fisiología, Esc. Medicina, Univ. de Concepción, Concepción, Chile).*

The effects of gamma Resorcilic acid, Butazolidin and Aspirin on basal O_2 consumption of a chilean toad were studied.

Normal toads show an increase in the basal O_2 consumption under the action of the three drugs, which is statistically significant with respect to the control series.

Decerebrate toads show also a significant increase.

Hypophysectomized toads show a less marked response when the above mentioned drugs are given.

The least appreciable effect of these antirrheumatic drugs was found in adrenalectomized toads. However, Butazolidin produced the highest relative increase in this series of experiments.

Effect of voluntary intake of ethanol on the survival time of rats submitted to chronic intoxication with carbon tetrachloride. J. MARDONES, J. CEMBRANO AND E. MUÑOZ. *(Inst. Farmacología, Univ. Chile, Santiago, Chile).*

Four groups of six rats each, fed with stock diet were submitted to chronic intoxication with carbon tetrachloride by daily exposure during one minute to an atmosphere saturated with vapors of this substance. Two of these groups of rats belonged to the "drinker" and the others to the "non-drinker" strain. The rats were housed in individual cages and one of the "drinker" groups and another of the

"non-drinker" ones were offered free choice of distilled water and a 10 % V. V. ethanol solution. The remainder groups received only distilled water.

During the experiment the "non-drinker" rats increased the voluntary alcohol intake while the "drinker" rats did not modify their alcohol consumption.

No influence of the alcohol intake on the survival time of the rats of the "non-drinker" strain was observed; but the survival time of the rats of the "drinker" strain which received alcohol was significantly higher than both the two "non-drinker" groups and the "drinker" one which did not receive alcohol.

Effects of intraventricular administration of acetylcholine, adrenaline and d-tubocurarine in the rat. R. F. MARSEILLAN AND A. P. CORRADO. *(Deptos. Fisiología y Farmacología, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

The motor, vegetative and behavioural effects produced by injection of acetylcholine, adrenaline, and d-tubocurarine in the lateral ventricle were studied in the white rat.

A simplified technique of permanent cannulation was developed. The cannula is made with a 2.38 mm round head brass screw, 2 mm long, in which a 1 mm hole is drilled for inserting a PE 50 polyethylene tube, cemented with epoxy resin. The cannula is implanted in a tapped hole at the level of the coronal suture, 1-1.5 mm from the midline. The tip of the polyethylene tube extends 3.8 mm below the calvaria. All drugs used were dissolved in normal saline, the volume injected being 0.05 ml or less; 0.05 ml of normal saline was used in the control injections. The animals were killed and their heads sectioned to verify the position of the cannula.

The results obtained with the three drugs tested —representatives of the wide group of substances experimented by Feldberg et al. in the cat— were similar to those described by the authors already mentioned.

d-tubocurarine produced immediately after the injection a state of excitation varying from increased motor activity to generalized convulsions. With a 5 μg dose the animal showed tachypnea, tremors, extension of the tail, mastication, and restlessness; one to three minutes afterwards, clonic movements of a periodic character appeared in one of the legs. Some animals had with this dose generalized seizures with complete recovery afterwards. Larger doses, 10 μg or more, always resulted in intense and sustained tonic-clonic generalized convulsions.

The injection of *acetylcholine*, from 30 to 50 μg, developed an immediate state of depression characterized by diminished motor activity and postural tonus, the animal lying on its side. Reaction to external stimuli was lessened. After some minutes, the rat passed through a phase of catatonia of variable intensity; in this condition, it could be placed and remained in the most absurd positions.

No effect was observed with 10 to 30 μg of *adrenaline*. When larger doses were injected (30-100 μg), the animal appeared depressed, with reduced reflex and motor activity and in some animals signs of a light hypnosis. The anesthesia-like condition described by Feldberg et al. in the cat did not appear in the rat.

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Bradykininogen concentration in plasma of women during the labor. A. R. MARTÍNEZ, F. CARVALHO AND R. DINIZ. *(Depto. Ginecología y Obstetricia, Medicina e Farmacología, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

Activation of proteolytic enzymes during the pregnancy and labor has been already demonstrated (Gilman, 1958). Bradykinin, a pain producing, oxytocic and vasoactive substance is liberated by proteolytic activity from an inactive precursor, bradykininogen, by enzymes showing the specificity of trypsin (Rocha e Silva, 1949). Alterations of bradykininogen concentrations in blood during labor may serve as an indirect evidence of bradykinin liberation or enhanced proteolytic activity in this condition.

The bradykininogen concentration in the plasma of 20 normal women was determined during the labor period by the method described by Diniz et al. (1961). The average value found in the plasma collected during

the first period of labor (A) was 21.6 ± 1.0 units/ml plasma, in the expulsion period (B) 16.1 ± 0.8 units/ml and in the puerperium (C), 20.9 ± 1.0 units/ml.

The mean difference (paired values) A-B, 4.9 ± 0.67 and C-B, 4.1 ± 0.69 units/ml were highly significant ($P < 0.001$). The difference A-C was not significant. In 3 cases where the fetus has been extracted by cesarean operation no difference was detected in bradykininogen concentration before and after the operation.

The average concentration of bradykininogen in the blood from the umbilical cord was 8.5 ± 0.4 units/ml.

New "in vivo" method for the determination of the disintegration time of medicaments administered orally. E. MONTUORI AND C. R. ORDÓÑEZ. *(Depto. Investigaciones, Laboratorio Dr. Gador y Cía., Buenos Aires, Argentina).*

The velocity of absorption of drugs administered orally is conditioned in the first place on their disintegration time in the gastroenteric duct; solubilization and further absorption depending on disintegration. So far, no routine methods for the control of disintegration time of drugs of prolonged action are available.

The Aa. suggest the addition of small quantities of PABA (5 to 19 mg) to the medicament as a kind of indicator. This substance appears in urine 10 minutes after ingestion and is completely eliminated after 90 minutes, a fact which permits to deduce the start and ending of the disintegration of a medicament.

A simple chromatographic method for the determination of small quantities of PABA in the urine is described.

Tentativa de purificação de frações biològicamente ativas da peptona comercial. A. G. A. NEVES. *(Depto. Bioquímica, Fac. Medicina, Univ. Minas Gerais, Belo Horizonte, MG, Brasil).*

Influence of age and sex upon sensitivity to procaine in rats. C. PAEILE, S. GUERRERO, I. CAMPOS AND C. MUÑOZ. *(Inst. Farmacología, Univ. Chile, Santiago, Chile).*

It was reported in a previous paper (Muñoz, C. *et al.*; Toxicol. & Appl. Pharmacol., 1961, 3, 445), that male rats are more resistant than females to the toxic effect of i.p. injected procaine. This difference was consistent with a higher procaine hydrolytic activity found in male liver as compared with that of females. Nevertheless, no differences in the mean lethal dose (MLD) of procaine in young rats of both sexes (under 45 days of age) receiving the drug intraperitoneally with the same rate as the adults were observed, while at that age the liver procainesterase activity was significantly higher in males.

In the present study the MLD of i.p. injected procaine in young (30 days) and adult rats of both sexes were compared by adjusting the injection rate to 2.0 mg per 100 g of body weight per minute. Our results showed that although in these experimental conditions the MLD of procaine in young animals was lower than in the adults, it was not significantly different in males and females. On the other hand, in adult rats previously treated with an esterase inhibitor, SKF 525-A (50 mg/kg) a marked decrease in the MLD of procaine was observed and the sexual difference to the toxicity of this drug disappeared (MLD: 223 ± 7.9 mg/kg in males and 241 ±4.9 mg/kg in females).

These results show that the sexual differences in the toxicity of procaine in rats appears to be only partially related with differences in the procaine hydrolytic activity of the liver and thus other involved factors deserve further investigation.

Nalorphine metabolism in rats. ANA PENNA-HERREROS, E. MORAGA AND J. MARDONES. *(Inst. Farmacología, Univ. Chile, Santiago, Chile).*

Two chromatographic methods for the separation of morphine and normorphine, one suggested by Axelrod (isoamil alcohol, n-butyl ether, water) and other developed in this Institute (pyridine, ethyl acetate, water) were proved to be useful to separate also nalorphine.

The incubation of nalorphine in the presence of rat liver homogenates showed that nalorphine appears to be rapidly coupled with a substance of the homogenates, since it could not be removed by the commonly used solvents. The incubation during 3 hours at 37°C

in the presence of oxygen released the nalorphine. Morphine and normorphine compete with nalorphine in this coupling reaction, since in the presence of any of these alkaloids, nalorphine can be removed from the homogenate. By incubating nalorphine with rat liver homogenates, part of it is transformed in normorphine, which could be recognized by chromatography. Brain homogenates neither coupled nor dealkylated nalorphine.

No recognizable amounts of nalorphine were found either in the liver or in the brain of rats which received doses as high as 50 mg/kg subcutaneously or 15 mg/kg intravenously, but in some experiments normorphine was found in the liver but not in the brain. In the liver of rats which received 50 mg/kg of nalorphine, this alkaloid as well as normorphine were found, but neither normorphine, nor nalorphine were found in the brain.

5-hydroxytryptamine sensitization of rat seminal vesicles to adrenaline *. ZULEIKA P. PICARELLI, NEIDE HYPPOLITO AND J. R. VALLE. *(Labor. Farmacología e Bioquímica, Esc. Paulista Medicina, São Paulo, Brasil).*

Longitudinal contractions produced by adrenaline (0.1-2.0 μg/ml) on normal rat seminal vesicles, maintained in a muscle chamber containing 7 ml of special nutritive solution (NaCl, 8.0 g; KCl, 0.42 g; $CaCl_2$, 0.20 g; NaH_2PO_4, 0.05 g; $NaHCO_3$, 1.25 g; dextrose, 1.0 g and distilled water to 1.1), aerated and kept at 30°C, recorded with a frontal lever giving sixfold magnification and with a load of 1-1.5 g, were greater when in presence of 5-hydroxytryptamine (5HT) (1-10 μg/ml). This sensitization was not observed for any other drug from those assayed, with exception of noradrenaline.

The antioxidant action of 5TH to which Lecomte (1) and Savini (2) have attributed similar results obtained in other pharmacological preparations, did not justify the sensitization. *Versene* (disodium salt of ethylenediamine tetraacetic acid) in a concentration of 10 μg/ml, enough for complete blocking "in vitro" autooxidation of adrenaline, had no influence on the increased response of this drug after 5HT.

The phenomenon could be due to inhibition of monoamine oxidase (MAO); actually β-phenylisopropylhydrazine, a potent inhibitor of this enzyme, increased the effect of adrenaline by itself and after it, there was no further sensitization by 5HT. But *iproniazid* has not produced the same effect and what is more significant, tryptamine also a good substrate of MAO, did not affect adrenaline contractions on the test preparation.

It seems probable that the sensitization of seminal vesicles to adrenaline is linked with inhibition of catechol-O-methyltransferase by 5HT, already observed by Bacq et al. (3) with other phenolic antioxidant substances.

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Crossed tachyphylaxis between morphine and meperidine, methadone and dextromoramide in rats. R. PRIETO, J. ALDUNATE, S. CODNER AND J. MARDONES. *(Inst. Farmacología, Univ. Chile, Santiago, Chile).*

The authors reported in a previous paper (Arzneimittel-Forsch, 1961, *11*, 665). that the intravenous administration of morphine to rats under urethane anesthesia induced, about 4 seconds after the injection, bradycardia and apnoea through a reflex mechanism initiated by the stimulation of vagal receptors located in the lungs. The intensity of the response increased with the logarithm of the dose and exhibited tachyphylaxis.

In the present paper, tachyphylaxis with two consecutive doses of methadone, dextromoramide or meperidine as well as crossed tachyphylaxis with morphine used as the first or the second dose were studied. EKG and respiration were continuously recorded. The degree of tachyphylaxis was evaluated according to the procedure mentioned in the previous paper (loc. cit).

Tachyphylaxis induced by methadone was lesser than that of morphine. The crossed tachyphylaxis between morphine and methadone, using morphine as the first dose, was similar to that observed between two doses of

morphine. When morphine was used as the second dose, tachyphylaxis was similar to that observed between two doses of methadone.

Tachyphylaxis with two doses of dextromoramide was small for the bradycardia and of medium degree for the apnoea. When morphine was the first injection, the tachyphylaxis revealed with dextromoramide was similar to that obtained between two doses of morphine; but it was of a lesser degree when dextromoramide was administered as the first dose and morphine as the second one.

The results of the experiments using two doses of meperidine were so irregular that it was impossible to establish whether or not any tachyphylaxis really exists. The crossed tachyphylaxis with morphine as the first dose was small in bradycardia and almost complete in apnoea. When morphine was injected as the second dose, no tachyphylaxis was observed and even the bradycardia and duration of apnoea were increased, suggesting that instead of tachyphylaxis a synergism between both drugs was present.

Anaphylactic reaction "in vitro" and anaerobic metabolism. A. PROUVOST-DANON AND H. MOUSSATCHÉ. *(Div. Fisiologia, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil).*

It has been shown that histamine release in the anaphylactic reaction is inhibited by oxygen lack (1, 2, 3). Uncouplers of oxidative phosphorylation such as 2.4-dinitrophenol, inhibit histamine release (4). It could be expected that the energy generated by the anaerobic cycle, in the same way as happens in the aerobic metabolism, would be available for the histamine release process. Results obtained on the effect of glycolytic metabolism upon the histamine release from guinea-pig and rat lung sensitized to horse serum, are summarized in this report.

Under anaerobic conditions, in the absence of glycolytic activity, there is a complete inhibition of histamine release from guinea-pig and rat lung by antigen. When glucose is present and anaerobic glycolysis is proceeding, inhibition is reversed, histamine being released by the antigen (5). Inhibitors of glycolysis such as sodium monoiodoacetate (10^{-3} M) and sodium fluoride (10^{-2} M) inhibit completely the release in the presence of glucose, while 2.4-dinitrophenol (10^{-4} M), uncoupler of oxidative phosphorylation, but not of anaerobic phosphorylation (6), has no effect upon it.

These results suggest that histamine release process during the anaphylactic reaction "in vitro" depends upon energy and that this energy can be furnished either by aerobic or by anaerobic metabolism.

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* *Fellow from the "Conselho Nacional de Pesquisas".*

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Inibição dos efeitos da neurocinina do líqüido cérebrospinal no duodeno e na pressão arterial do rato. L. RAMOS, L. F. CHAPMAN, T. ZILELI E A. O. RAMOS. *(Depto. Farmacologia, Fac. Medicina, Univ. São Paulo, Brasil e Neurology Dept. Cornell Univ., New York, N. Y., U.S.A.).*

Chapman e col. (1960, 1961) e Ramos (1961) descreveram a neurocinina, polipeptídeo que corre durante a vasodilatação reflexa da pele e no líqüido cérebrospinal. Além de poder conter o próprio polipeptídeo, o líqüido cérebrospinal (LCS) contém uma enzima capaz de formá-lo quando em contado com globulinas do plasma.

A neurocinina usada no presente trabalho foi preparada a partir da incubação do LCE com gama-globulina bovina, fração II (NBC).

A neurocinina é droga hipotensora no rato e produz relaxamento do duodeno isolado dêsse animal.

A administração de hidrocortisona (100 mg/kg) impediu a manifestação dos efeitos hipotensores da neurocinina. Quando aplicada ao banho de órgão isolado na concentração de 0.33 mg/ml a hidrocortisona inibiu os efeitos relaxantes da neurocinina sôbre o duodeno de rato.

O salicilato de sódio (200 mg/kg) também inibiu os efeitos da neurocinina na pressão arterial.

Quando aplicado ao banho do duodeno isolado de rato, o salicilato de sódio (1 a 3) mg/ml) produziu intenso relaxamento e sòmente após lavagens sucessivas é que o órgão voltou ao tono anterior. Entretanto, mesmo após restabelecido o tono do duodeno, a neurocinina se encontrava inibida.

(Este trabalho foi em parte auxiliado pela Society for the Investigation of Human Ecology.)

Atividade farmacológica do humor aquoso. L. RAMOS E A. O. RAMOS. *(Depto. Farmacologia, Fac. Medicina, Univ. São Paulo, Brasil).*

O humor aquoso (HA) do cão, do gato e do boi, é capaz de relaxar o duodeno isolado do rato e de contrair o íleo do cobaio e o útero de rato. O humor aquoso é capaz de provocar hipotensão no galo e no rato, sendo porém inativo no cão e no gato.

A fervura em banho-maria não altera a atividade do HA no duodeno de rato, porém reduz acentuadamente o seu efeito hipotensor (no galo e no rato). A atividade do HA é precipitável pela acetona.

O HA dialisado conserva a propriedade de produzir hipotensão, porém o seu efeito no duodeno de rato é invertido, pois torna-se estimulante.

A incubação do HA com tripsina, sôro humano ou quimotripsina causa o desaparecimiento da sua atividade sôbre o duodeno.

Os resultados iniciais indicam a existência no HA de dois fatôres farmacològicamente ativos; um fator (nº 1) relaxante do duodeno do rato, dialisável, lábil à incubação com tripsina, quimotripsina ou sôro, e termostável.

O outro fator (nº 2) é hipotensor, não dialisável e termostável.

(Este trabalho foi em parte auxiliado pela Society for the Investigation of Human Ecology.)

Comparação entre bradicinina pura e padrão. M. ROCHA E SILVA, C. R. DINIZ E A. ANTONIO. *(Depto. Farmacologia, Fac. Medicina, Univ. São Paulo, Rib. Prêto, SP, Brasil).*

Inhibition of the Shwartzman reaction by antagonists of histamine and serotonin *. G. ROSENFELD. *(Labor. Hematologia, Instituto Butantan, São Paulo, Brasil).*

The thrombo-hemorrhagic lesion, characteristic to the local Shwartzman reaction induced in rabbits by injecting toxin of *Serratia marcescens*, was completely inhibited when antihistaminic derivatives from Phenothiazine as Fenergan, Ampliactil and Neozine were injected intradermically at the sensitized place. The intravenous injection of the same substances inhibited the reaction only in some of the animals. Serotonin antagonists like LSD, BOL and UML also prevented the reaction in some of the animals when injected intradermically. All injections were given 15 minutes before the provoking dose of toxin.

Since antihistaminics also have an antiserotonin activity, there is a confirmation of the function of serotonin in the production of the local lesion of the Shwartzman reaction; however the participation of histamine cannot be eliminated.

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Glucose metabolism and histamine release by antigen and by anaphylatoxin "in vitro". A. M. ROTHSCHILD. *(Depto. Farmacologia, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

Previous work (1) from this laboratory has shown that in the isolated rat diaphragm, histamine release is associated with cellular energy metabolism, since it has been observed that the inhibition of the histamine-releasing action of compound 48/80 by agents which block oxidative phosphorylation (dinitrophenol (DNP), anoxia, sodium cyanide, salicylate, etc.), was abolished by glucose, but not by xylose, pyruvate, succinate or other intermediates of the Krebs cycle.

It was considered of interest to verify whether similar factors controlled the release of histamine from sensitized tissue by antigen and

from normal tissue by anaphylatoxin. It could be shown that the release of histamine obtained when antigen was added to pieces of isolated, sensitized rat diaphragm, was readily inhibited by DNP or cyanide, but that this inhibition was suppressed in the presence of glucose. Similar results were obtained when the action of these inhibitors, as well as that of anoxia was tested on the release of histamine induced by antigen acting on sensitized guinea-pig lung slices. The action of anaphylatoxin, a potent histamine releaser in the guinea-pig (2), was similarly affected. Since previous work (3) has shown that anaphylatoxin depletes guinea-pig lung histamine stores (2), degranulates mast cells (4), desensitizes guinea-pigs to anaphylactic shock (5) and is related to complement fixation reactions (6), the finding that histamine release by anaphylatoxin takes place under apparently similar metabolic conditions as the release of antigen, yields further support to the theory (2) that anaphylatoxin or a similar factor is involved in histamine release following antigen-antibody interaction in animal tissue.

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Farmacología de la eyección láctea en la mujer. N. L. SALA Y Y. SICA-BLANCO. *(Serv. Fisiología Obstétrica, Fac. Medicina, Montevideo, Uruguay).*

Relación dosis efecto en la tira de glándula mamaria de coneja *in vitro* con la ocitocina. G. STRADA-SÁENZ, L. A. BRANDA Y V. H. GONZÁLEZ-PANIZZA. *(Serv. Fisiología Ostétrica, Fac. Medicina, Montevideo, Uruguay).*

Ação neuromuscular da peçonha de *Micrurus*. O. VITAL-BRASIL. *(Depto. Farmacologia, Fac. Medicina, Univ. São Paulo ,São Paulo, Brasil).*

A peçonha das corais (*Micrurus, Elapidae*) produz bloqueio neuromuscular na preparaçao frênico-diafragma isolado do rato. A neostigmina exerce um certo efeito antagônico, restabelecendo, de um modo parcial e transitório, a transmissão dos impulsos nervosos.

No diafragma desnervado isolado do rato, a peçonha das corais abole, com extraordinária potência e grande seletividade, a resposta (contratura) provocada pela acetilcolina, pela nicotina ou pelo decametônio. A depressão das contrações do diafragma desnervado isolado à excitação elétrica, causada pela nicotina ou pelo decametônio, é também suprimida pela peçõnha. O bloqueio da ação estimulante da acetilcolina, da nicotina ou do decametônio não parece possa ser imputado à atividade de nenhuma das enzimas ofídicas conhecidas.

Os resultados acima resumidos sugerem que o bloqueio neuromuscular produzido pela peçõnha das elapídias decorra de estabilização da membrana das placas terminais à ação despolarizante da acetilcolina.

As peçonhas de algunas crotalídias sulamericanas (*Crotalus terrificus terrificus, Bothrops jararacussu, B. atrox*) encerram componentes de ação farmacológica semelhante à das corais no diafragma desnervado isolado. A potência bloqueadora da ação estimulante da acetilcolina, exibida por essas peçonhas, é, porém, muito menor do que a da peçonha de *Micrurus*. As peçonhas da *B. jararaca*, da *B. alternata*, da *B. cotiara* e da *Vipera aspis* mostraram-se inativas no diafragma desnervado isolato do rato.

Liberação de histamina do diafragma isolado de rato pelo composto 48/80. I. VUGMAN. *(Depto. Farmacologia, Fac. Medicina, Univ. São Paulo, Ribeirão Prêto, SP, Brasil).*

Neste trabalho foi estudada a distribuição da histamina e dos mastócitos no diafragma tendo sido confirmada a existência de histamina nos dois hemidiafragmas (1) e também verificada a sua distribuição irregular dentro de cada um dêles. Os mastócitos apresentaram-se dispostos, tanto na face torácica como na abdominal, em dois planos, ambos bastante su-

perficiais; no mais profundo dispunham-se ao longo das fibras musculares e no mais superficial dispunham-se de maneira irregular, ligados à serosa de revestimento, concentrando-se especialmente na inserção dos ligamentos e no tecido conjuntivo que envolve o nervo frênico. Uma correlação entre o teor de histamina do diafragma e o pêso dos ratos usados nestas experiências (80 a 300 g) mostrou que, dentro dêstes limites de pêso, o teor de histamina manteve-se pràticamente constante apresentando uma leve redução com o aumento de pêso. A verificação da possível influência que o teor de histamina do tecido exerce sôbre a quantidade liberada mostrou que a porcentagem de histamina liberada independe do teor existente.

No estudo de condições que podem influir na libertação de histamina pelo composto 48/80 verificou-se que a ausência de cálcio no líqüido de incubação (solução fisiológica-tampão fosfato, pH 7.3), reduziu significativamente a quantidade de histamina liberada. Em pH 6.4 a ausência ou presença dêste ion não afetou a liberação. O tratamento prévio do diafragma com verseno aboliu quase totalmente a liberação de histamina. A adição de cálcio após o verseno permitiu uma recuperação parcial da liberação.

A adição de glicose ao líquido de incubação aumentou de maneira significativa a liberação de histamina, sendo que o jejum prévio não exerceu qualquer influência.

Uma comparação feita entre a incubação em solução fisiológica-tampão fosfato-cálcio e o líqüido de Krebs Ringer-fosfato mostrou que não há diferença nos níveis de liberação de histamina em ambos os meios.

A influência do pH foi estudada com o auxílio de dois sistemas tampões: fosfato e Tris-HCl. O primeiro nos pHs 5.5, 6.4 e 7.2 e o segundo nos pHs 6.9, 7.4 e 8.2. Houve redução acentuada na liberação em 5.5 e 6.4 e um nível constante de liberação máxima entre 7.2 e 7.9 com uma pequena queda em 8.2.

A influência da concentração do composto 48/80 foi estudada em duas experiências. A primeira com as concentrações de 40, 20, 10 e 5 μg/ml mostrou haver um nível máximo de liberação em 40 e 20 μg com uma redução significativa nas concentrações menores. A segunda com 20, 5, 1, 0.2 e 0.04 μg/ml confirmou os resultados da primeira experiência para os níveis de 20 e 5 μg e mostrou a existência de uma queda muito acentuada entre 5 e 1 μg e de ser pràticamente nula a liberação com 0.2 e 0.04 μg.

A incubação de diafragmas em 36°, 38° e 42°C em presença de 48/80 mostrou que, dentro dêstes limites, a variação da temperatura não alterou significativamente a liberação de histamina. O pré-aquecimento por 25 minutos em temperaturas de 42.5° a 45°C levou a uma inibição progressiva da liberação que foi nula a 45°C. O pré-aquecimento a 45°C por 5 minutos reduziu de 8 por cento, por 10 minutos de 18 por cento e por 20 e 40 minutos aboliu totalmente a liberação de histamina. A liberação espontânea a 45°C não diferiu da que ocorre a 37°C. Ao contrário do que ocorre com a inibição da liberação por substâncias como dinitrofenol e cianeto (2), a que é causada pelo pré-aquecimento a 45°C não foi revertida pela adição de glicose ao líqüido de incubação.

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Inhibition of gastric acid secretion by compound 48/80 in the rat. J. Weinberg and M. L. Horta. *(Depto. Fisiologia, Fac. Medicina, UMG, Belo Horizonte, Brasil).*

The stimulation of gastric acid secretion by compound 48/80, a potent histamine liberator, was shown by Paton and Schachter (1951) in the dog and Smith (1953) in the cat. As this compound liberates histamine in the rat (Feldberg and Talesnik, 1953), we decided to study its action on the gastric acid secretion of this animal. We used Wistar strain albino rats of both sexes and the experiments were performed according to the method described by Shay *et al.* (1954) modified. The rats were divided into groups to be injected with compound 48/80, histamine and saline (control group). Gastric acidity was measured in ml of NaOH 0.01 N used in titration and the value found was calculated for 100 g of body weight. The group injected with histamine showed an increase of 81 % in gastric acidity when compared with controls. Compound 48/80, injected in doses of 25, 50, 75 and 100 μg/100 g caused a decrease in gastric acidity, which varied from 39 to 90 %, according to the dose. This inhibitory

effect of 48/80 on the gastric acid secretion in the rat perhaps can be explained by the antihistaminic or atropine-like action of this compound. This interpretation finds support in the observation (Paton, 1951) that compound 48/80 shows an antihistaminic and atropine-like action on the guinea-pig's isolated ileum.

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Importância do cálcio na sensibilização pela eserina e pela neostigmina da resposta do músculo reto abdominal do sapo à acetilcolina. E. XAVIER. *(Lab. Farmocolgía e Bioquímica, Esc. Paulista Medicina, São Paulo, Brasil).*

Ao se estudar a sensibilização pela eserina e pela neostigmina da resposta do músculo reto abdominal do sapo (*Bufo ictericus*, Spix) à acetilcolina (1) foi verificada a importância do teor de cálcio no líqüido nutritivo empregado. Nas experiências anteriores usamos o líqüido de Ringer para batráquio com a seguinte composição milimolar: NaCl 109, KCl, 4.02, $CaCl_2$ 1.58, $NaHCO_3$ 4.25 e glicose 3.96 (pH 7.4 μ 0.122). Nas experiências ora descritas variamos o teôr de cálcio para mais ou menos, tendo o cuidado de manter os líqüidos nutritivos, pràticamente, de mesma fôrça iônica e pH.

Com Ringer pobre em cálcio (0.39 em vez de 1.58 milimolar) a preparação se mostrou mais sensível à acetilcolina, cujas concentrações ativas passam, em média, de 0.04 para 0.01 μg/ml. Nessas condições, a ação sensibilizante da eserina permanece a mesma mas a da neostigmina desaparece a esta droga passa a bloquear parcialmente a resposta acetilcolínica.

O Ringer rico em cálcio (2.61 em vez de 1.58 milimolar) diminui a sensibilidade da preparação que responde a concentrações de acetilcolina de 0.05-0.02 μg/ml. Há um decréscimo proporcional do efeito sensibilizador da eserina, enquanto a neostigmina determina um aumento de sensibilidade de cêrca de 15 vêzes. A eserina e a neostigmina foram usadas no Ringer normal e com maior teor de cálcio nas concentrações de 10 a 0.1 μg/ml. No caso do líqüido nutridor pobre em cálcio foi necessário diminuir a concentração de eserina dado o aparecimiento de resposta muscular, sendo mantida pràticamente a mesma concentração de neostigmina.

Cicardo, em 1941 (2), tinha estudado no sapo, a importância dos iônios cálcio e potássio para a contração dos músculos estriados. Bülbring et al. (3) observaram um aumento da reatividade de músculos estriados da rã, imersos em Ringer pobre em cálcio, na dependência de perturbações do potencial de membrana do miônio. Frank (4) também chamou a atenção para a importância do cálcio como ativador das miofibrilas.

Os resultados aqui descritos mostram que a sensibilidade do reto abdominal do sapo à acetilcolina depende de uma concentração ótima de iônios Ca^{++}. No Ringer pobre em cálcio, o músculo torna-se mais sensível à acetilcolina, o efeito da eserina não se modifica, mas a sensibilização pela neostigmina desaparece, diminuindo inclusive a resposta à acetilcolina. Por outro lado, o aumento do teôr de cálcio reduz a sensibilidade à acetilcolina e à eserina, mas, paradoxalmente, favorece os efeitos da neostigmina.

Êstes resultados indicam que a eserina e neostigmina melhoram a resposta do reto abdominal do sapo à acetilcolina por mecanismos diferentes.

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Effects of anephrotensin on the liberation of ACTH by the hypophysis in rats. B. ZAMORANO, H. CROXATTO AND S. BAZAES. *(Lab. Fisiología, Fac. de Filosofía y Educación, Univ. Chile, Santiago, Chile).*

Experiments were carried out in order to investigate whether preparations of anephro-

tensin obtained from rat and dog serum were able to bring a release of ACTH by the hypophysis. For this purpose adult rats were used. Changes in the ascorbic acid contents of adrenals of these same rats were determined. The first group was injected with a dose of purified sample of anephrotensin; the control group was injected with solvent solution and a third group was injected with serotonin, which as Moussatche and Pereira (1957) established, produces a decrease of the ascorbic acid. Besides, other animals were injected with several doses of bradykinin. The results and statistical estimates show that anephrotensin as well as bradykinin (5 μg) induce a significant decrease of the ascorbic acid contents of the adrenal. The effect obtained with anephrotensin is completely prevented when hypophysectomy is performed 24 hours before the experiment.

FISIOLOGÍA COMPARADA
COMPARATIVE PHYSIOLOGY

Modificaciones de la actividad espontánea del sistema nervioso de insecto por estimulaciones aferentes. L. AGUILAR Y J. V. LUCO. *(Univ. San Marcos, Perú; y Lab. Neurofisiología, Univ. Católica Chile, Santiago, Chile).*

Se parte de la observación que la mencionada actividad tiene una frecuencia relativamente estable y que puede ser modificada tanto en un sentido de disminución como de aumento por un estímulo eléctrico repetitivo aplicado a una vía aferente, dependiendo dicho sentido de la duración del tren aplicado. Tal modificación de la frecuencia es relativamente persistente y su declinación ocurre excediendo los límites de una simple post-descarga.

Valiéndose de una preparación de ganglio *in vitro*, se procede a retirar parcialmente la vaina conectiva que lo rodea y se hacen registros intracelulares obteniéndose distintos patrones de actividad que son relacionados tanto con los estímulos aplicados como con los registros extra-celulares obtenidos simultáneamente en un intento de aclarar el o los mecanismos que mantienen la actividad espontánea en un centro nervioso de la complejidad del ganglio de insecto.

Estudio electrofisiológico de la habituación y de la deshabituación de la reacción de huída de la cucaracha, *Blatta orientalis* y *Blatta germánica*. A. DAVIDOVITCH Y C. MARTINOYA. *(Lab. Neurofisiología, Univ. Católica Chile, Santiago, Chile).*

Se investigó la reacción conductal de la cucaracha sometida a una mínima disección, con el objeto de preservar su integridad estructural y funcional. El animal, situado dentro de una cámara silente, se fijó con sus patas libres. Se registró la actividad eléctrica de los músculos toráxicos, que intervienen en la reacción de huída, como respuesta de la vía final común, a estímulos naturales —soplos de aire, luz, sonidos— y eléctricos en los nervios sensitivos de los cercos.

La estimulación con un ritmo regular produce, con los estímulos naturales (probablemente no máximos) y con los eléctricos submáximos, una disminución gradual de la respuesta hasta su desaparición. Con los estímulos eléctricos máximos se determina una intensa disminución, pero sin llegar a la abolición total. La respuesta reaparece: a) Después de un reposo; b) Por aumento de la intensidad del estímulo; c) Por una extraestimulación única o repetida, de la misma u otra modalidad sensorial, intercalada entre dos estímulos regulares. El extraestímulo puede, para este objeto, ser efectivo, aunque no dé respuesta. d) Por la aplicación simultánea del estímulo que ya no es efectivo con otro que todavía lo es. e) Por la aplicación simultánea de dos estímulos no efectivos debido a la repetición.

Una vez desaparecida la respuesta, la continuación prolongada de la estimulación, puede llevar a una supresión de la respuesta a los demás estímulos.

Se trata de un fenómeno acumulativo, porque al repetirse los ensayos, se observa que se requiere reposos más largos para la reaparición de la respuesta y que el decremento y desaparición de la respuesta, se consigue cada vez con un menor número de estímulos. El fenómeno tiene características de habituación y se le puede diferenciar claramente de fatiga o de adaptación de receptor.

En otra serie experimental, se ha estudiado esta reacción conductual en cucarachas decapitadas. También se observa el decremento

de la respuesta, resultado que indicaría un rol no esencial en el mecanismo de este fenómeno, de las funciones inhibidoras del ganglio supraesofágico.

Fine structure of snail's neurones. J. FERNÁNDEZ HIDALGO. *(Inst. Fisiología, Esc. Medicina, Univ. Chile, Santiago, Chile).*

Visceral ganglia and intestinal nerves of Helix fixed in osmium tetroxide have been studied with the electron microscope.

Neurones and proximal axon segments are covered with a variable number of glia expansions forming irregular palisades.

Neuron's cytoplasm shows endoplasmic reticulum, Golgi material, lipoid droplets and secretion granules showing internal structure. Cellular and axonic membranes show plenty of invaginations that push in the underlying glia.

Neuropile display the profiles of three types of fibers: a) Giant axons with neurofibrillar material and axolemic invaginations; b) Median caliber fibers with dense granules or vesicular content; c) Synaptic endings on giant axons. These axo-axonic endings show a presynaptic element with mitochondriae and varied synaptic vesicles in relation to its structure and size (300-1500 angstrom). In addition, glia nuclei and flat glia expansions covering some fibers have been seen.

In the intestinal nerves the axons appear surrounded by typical glia palisades. The inner one of them is also pushed in at the level of the axonic invaginations.

Spontaneous activity, excitation and inhibition in snail neurons. B. HOLMGREN AND S. FRENK. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

It has been demonstrated that the neuron cells of snail ganglia are easily accessible (1); we have initiated the study of the spontaneous and provoked electrical activity in these cells, considering that the electrical activity of these neurons can reflect, in some way, the global process of the ganglia.

The periesophageal gangliar ring of garden snails was dissected maintaining intact the connections of the ganglia with the paleal nerves which innervate the mantle. The preparation was mounted in a lucite camera that had platinum electrodes to stimulate the nerves. The ganglia were stripped of their conjuctive coverings and ganglion cells were exposed (100 - 300 u). The register was done with micropipettes filled with KCl (3 M) coupled with a DC amplifier and an oscillograph.

The spontaneous activity was very irregular and went from a constant discharge (frequency 20 c.p.s.) to burst of irregular rates; potentials of different sizes appear sometimes (abortive spikes) and oscillations of the membrane potential.

In some cells, stimulation of the nerves produced evoked spikes whose latency varied with the frequency of stimulation (due to problems of fatigue). Another type of cell presented, with repeated stimulation, a double propagated potential. Hyperpolarizing potentials were also observed in certain types of cells.

In other cells, repeated stimulation at low frequency, produced first a potential of depolarizing spike, and then the inversion of this potential in a hyperpolarizing potential provoking a complete inhibition of this cell.

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O ritmo de secreção das glândulas mandibulares de apídeos. J. O. NEDEL. *(Fac. Filosofia, Ciencias, Letras· Cristo-Rei, São Leopoldo, RGS, Brasil).*

O produto da secreção das glândulas mandibulares tem largo emprêgo entre os insetos sociais, na intercomunicação (abelhas, zângãos, formigas etc.). Estudos feitos, sôbre a formação, desta secreção em algumas espécies de apídeos, principalmente na espécie *Trigona postica* Latreille, mostraram que o produto glandular nesta espécie se regenera num período de mais ou menos 30 minutos. Isto corresponde ao tempo que a abelha normalmente precisa para ter à disposição quantidades maiores dessa secreção, depois de ter descoberto um manancial de pasto, segundo experiências de Kerr e Lindauer (1958).

Medições micrométricas de células em atividade secretora mostraram, que o núcleo das células aumenta em tamanho, antes de aparecer a secreção no citoplasma. Isto está em consonância com os dados da fisiologia celular, segundo os quais (Hass 1955, Brachet 1958) se sintetizam no núcleo as nucleoproteinas que atuarão como enzimas e coenzimas na síntese da secreção glandular no citoplasma.

Tendo as células glandulares em questão, uma estrutura bem diferente das células glandulares com que normalmente se fizeram estudos e fisiologia glandular (sem polaridade, sem complexo de Golgi, etc.), e sendo elas de tamanho relativamente grande e de ritmo fàcilmente controlável, possuimos nelas um objeto muito favorável para estudos de fisiologia glandular de invertebrados.

Sôbre a fisiologia das fibras musculares lisas de invertebrados. P. SAWAYA. *(Depto. Fisiologia Geral e Animal, Univ. São Paulo, São Paulo, Brasil).*

Sôbre substância ictiotóxica em material de ninho de abêlha selvagem. *(Arapuã — Trigona rufricus). (Dpto. Fisiologia Geral e Animal, Univ. São Paulo, São Paulo, Brasil).*

O trato intestinal, orgão respiratório de peixes. P. SAWAYA E L. M. DE PETRINI. *(Depto. Fisiologia Geral e Animal, Univ. São Paulo, Brasil).*

Os peixes que habitam regiões tropicais apresentam freqüentemente adaptações diversas para suprir o organismo com o oxigênio de que necessitam.

Krogh (1941), entre outros, registra tais adaptações no interessante capítulo denominado "Respiração de Emergência".

Trabalhamos com o peixe "cascudo" (*Plecostomus plecostomus* Linneu), bastante comum nos cursos d'agua que circundam a cidade de São Paulo, o qual apresenta aspectos muito interessantes no seu trato digestivo.

Já foram assinaladas a presença de uma verdadeira cloaca neste peixe e a ocorrência de respiração intestinal (Sawaya e De Petrini, 1960).

Continuando as pesquisas procuramos estudar a respiração que se efetua pelo estômago, característica desta espécie de cascudo.

O presente trabalho refere-se principalmente à determinação das regiões respiratória e digestiva do estômago. Em preparações microscópicas, na parte da pequena curvatura, próxima à região cardíaca do estômago, notamos a presença de numerosos capilares intra-epiteliais e ausência de glândulas. Estas distribuem-se na grande curvatura da região cardíaca e na região pilórica. Quer-nos parecer que o estômago, que nêsses peixes aparece sempre repleto de ar, concorre para a respiração através dos capilares intra-epiteliais acima referidos. A transição do epitélio do estômago para o duodeno é brusca, sendo a principal diferença a ocorrência de uma quantidade enorme de glândulas mucosas bem evidentes na mucosa duodenal.

A histofisiologia das diferentes partes do estômago e do duodeno será discutida.

Trabalho efetuado com o auxílio da Fundação Rockefeller.

HISTOLOGÍA
HISTOLOGY

Captación de I-131 por la tiroides humana. Estudio autoradiográfico. A. J. ANDRADA, R. DENDUKES, F. MÜLLER Y E. I. HERNÁNDEZ. *(Inst. Investigaciones Médicas, Univ. Buenos Aires, Hosp. Tornú, Buenos Aires, Argentina).*

La tiroides incorpora en forma fisiológica I-131 el que se comporta química y biológicamente como su isótopo; por medio de la autoradiografía es posible realizar, de acuerdo a la captación por el parénquima glandular, una representación gráfica de la topografía funcional de la tiroides. Para considerar las variaciones de dicha incorporación del Iodo se estudió un grupo de pacientes a los que se les administró una dosis de 2 mC de I-131, 24 horas antes de obtenerse material, que se hizo por biopsia a cielo abierto, punción o tiroidectomía. Mediante algunas modificaciones de la técnica del "Stripping film" se obtuvieron imágenes histológicas, que al compararlas en las distintas condiciones glandulares permiten afirmar que en la tiroides normal la

captación está en relación directa, aparte de la dosis y tiempo de exposición, con la presencia de tejido folicular y coloide.

La incorporación del isótopo no es uniforme y depende en general del tamaño folicular: a mayor tamaño, menor intensidad. Las variaciones de intensidad en folículos de igual tamaño apoyarían el concepto de "función folicular" explicable en virtud de las variaciones fásicas del metabolismo intrafolicular del I-131.

En las distintas tiroideopatías se determinó la correspondencia de la imagen historradiográfica con otros parámetros de la función tiroidea. Se comentarán estos resultados así como los obtenidos en un grupo de pacientes hipofisectomizados sin una repercusión tiroidea evidente.

Contribution to the study of active transport across epithelia. M. H. Burgos. *(Inst. Histología y Embriología, Fac. Ciencias Médicas, U.N.C., Mendoza, Argentina).*

The transport of colloidal particles across some epithelia of the hamster was studied with the electron microscope after the injection of colloidal mercuric sulphide. At the capillary endothelium level the particles are transported by vesicles of pinocytosis. In the *Rete testis* squamous epithelia, colloidal particles appear conglomerated inside deep invaginations of the cytoplasmic membrane, as has been described for macrophages.

Ductuli efferentes show ciliated and non ciliated cells, the latter only have colloidal particles between the microvilli and inside the cytoplasmic funnels and apical canaliculi continuous with the cell membrane.

These canaliculi open into vacuoles which appear to concentrate the particles in higher number. In the cells of the epididymis, sterocilia incorporate particles by pinocytosis along its length and between sterocilia the cytoplasmic membrane invaginates into funnels and canaliculi which also present colloidal particles. As in *Ductuli efferentes*, the large vacuoles concentrate particles into great clusters.

Contact between particles and cells is mediated by a substance of low electron density which is considered as a cellular "carrier". The nature of this "carrier" and the function of the Golgi complex in active transport, is discussed.

Comportamiento histoenzimológico (fosfatasa ácida y alcalina, succinodehidrogenasa y beta glucuronidasa) en heridas de piel y mucosa oral. R. L. Cabrini y F. A. H. Carranza. *(Depto. Biología, C. N. E. A., Buenos Aires, Argentina).*

Se ha estudiado el comportamiento de diferentes enzimas presentes en la piel y la mucosa oral durante el proceso de curación de heridas.

La fosfatasa ácida disminuye al nivel de la proliferación epitelial y sólo se regenera cuando se produce la queratinización. La succinodehidrogenasa también decae en zonas de proliferación epitelial. Por el contrario se observa un claro aumento de la beta-glucuronidasa en el epitelio proliferante. La fosfatasa alcalina parece no intervenir en la etapa de regeneración epitelial.

La regeneración del tejido conectivo se vincula a un aumento muy acentuado de fosfatasa alcalina, en menor grado se observa un aumento de fosfatasa ácida, beta-glucuronidasa y succinodehidrogenasa. Los granulomas de cuerpo extraño presentes en algunas heridas son particularmente ricos en fosfatasa ácida, succinodehidrogenasa y beta-glucuronidasa.

En cobayos escorbúticos encontramos una caída casi total de fosfatasa alcalina a nivel de la herida, la fosfatasa ácida por el contrario no disminuye y en algunas condiciones aumenta moderadamente..

Alteraciones histoquímicas producidas por altas dosis de rayos X en cabeza de ratas jóvenes. R. L. Cabrini, F. Schajowicz y F. A. H. Carranza. *(Depto. Biología, C. N. E. A., Buenos Aires, Argentina).*

La cabeza de ratas Wistar de 1 a 7 días de edad, fué expuesta a una dosis única de 16.000 rad, con una protección adecuada del cuerpo mediante un blindaje. Los animales se sacrificaron diariamente hasta los 7 días de la irradiación. Posteriormente se efectuó la de-

mostración histoquímica de la basofilia, glucógeno y fosfatasa alcalina en cortes adecuados como para poder observar la piel, la mucosa oral y hueso membranoso en osteogénesis.

Desde el primer día post-irradiación se observaron importantes variaciones en el comportamiento histoquímico de todas las substancias estudiadas, en especial de la basofilia. En general dichas variaciones estaban vinculadas con alteraciones morfológicas fáciles de controlar histológicamente.

Incorporação de amino-ácidos tritiados em nuclêicos. J. CARNEIRO. *(Lab. Fisiología Celular, Fac. Medicina São Paulo, Univ. São Paulo, Brasil).*

Citoanalizador: un nuevo instrumento óptico electrónico de investigación biológica. J. R. CORDERO-FUNES Y J. H. TRAMEZZANI. *(Lab. Neurobiología, Inst. Biología y Medicina Experimental, Obligado 2490, Buenos Aires, Argentina).*

Se describe un equipo óptico electrónico destinado a medir superficie de partículas de formas variables cuya área no sea menor de 10 micrones cuadrados.

Consta de un sistema óptico similar al de un microscopio para la observación y proyección aumentada convenientemente del espécimen sobre una pantalla, en la cual un rastreo ortogonal efectuado a intervalos idénticos proporciona información sobre la densidad óptica en cada punto. La medida se efectúa en una superficie de 16 x 16 puntos. Por medio de una cabeza fotométrica muy sensible se efectúa la medida seriada de todos los puntos y su información se analiza por un computador electrónico transistorizado que la registra en forma numérica. Las densidades son seleccionadas en doce niveles, variables a voluntad, memorizadas y vertidas en forma decimal a la máquina de escribir. Por la naturaleza de la mascarilla que se usa para el rastreo, cada punto registrado significa un área de un micrón cuadrado para esa densidad óptica (usando ampliación 1000x). Si se usan otros grados de ampliación se pueden medir otros tamaños; el límite inferior está fijado por el poder de resolución del microscopio.

El diseño se ha efectuado especialmente para la medida de núcleos y nucleolos y sus respectivas relaciones con el citoplasma, pero eventualmente puede usarse para la medida de granulaciones, partículas en suspensión, radioautografía, placas de microscopía electrónica, etc.

El funcionamiento enteramente automático elimina el factor humano en la precisión de la medida que según la muestra puede ser del orden del 1 % en cada una; asimismo permite un rendimiento muy elevado al llevar el tiempo de medida a 18 segundos aproximadamente, por núcleo.

Action of X-rays in different periods of the hair cycle in castrated C_3H mice. C. E. EPPER, A. B. HOUSSAY AND E. I. SAURER. *(Com. Nac. Energía Atómica de la República Argentina y Centro de Endocrinología, Godoy Cruz 1221, Buenos Aires, Argentina).*

Since in several pure strains of mice the histologic structure of hair follicles of the skin undergoes a simultaneous variation after castration, gonadectomized mice were used for the study of the effect of total and partial irradiation with X-rays.

Total or partial irradiation of mice with 200 rad one day after castration (hair follicles in telogen phase) cause a slight loss in pigment and a dose of 400 rad produces, besides a marked loss in pigment, a significant delay in hair growth. In animals irradiated with 200 or 400 rad 6 or 11 days after castration (hair follicles in anagen 2 or 6) there was no difference with control animals.

Partial irradiation with 800 or 1200 rad one day after castration causes an intense loss of pigment and a marked delay in hair growth. When partial irradiation is performed 6 days after castration the effects are less evident, whereas if the same doses are applied 11 days after castration only the 1200 rad dose elicits visible effects.

This experiment demonstrates the different radiosensitivity according to the phase of the hair cycle that is simultaneously induced by gonadectomy in the hair follicles.

Fracciones solubles del colágeno y proteínas relacionadas en conectivo dér-

mico de rata de diferentes edades. N. González-Cadavid, B. Denduchis y R. E. Mancini *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Aplicando el método de fraccionamiento de Jackson, con varias modificaciones sobre tejido conectivo dérmico normal de rata, se obtuvieron diversas fracciones solubles de colágeno y ciertas proteínas acuosas presumiblemente relacionadas. Ello permitió estudiar sus variaciones porcentuales relativas en función de la edad del animal, desde las etapas embrionarias a las adultas. Los datos hallados se comparan con los obtenidos por otros autores en el desarrollo del granuloma provocado experimentalmente, que ofrece algunas diferencias metabólicas con el de la piel normal.

Se observó una disminución porcentual gradual del total de los colágenos solubles, en conexión con el descenso de la síntesis fibrilar con la edad, que estaría vinculada con su presunto papel como precursores del colágeno.

Fueron analizadas las variaciones de los niveles respectivos de las fracciones neutrosolubles y ácido soluble, considerando a la primera como una etapa más reciente del metabolismo fibrilar. Los resultados confirman las teorías actuales que relacionan el envejecimiento de las fibras con la intensificación del número y fuerza "crosslinking" entre las macromoléculas del colágeno y la capacidad extractiva de las soluciones utilizadas.

Asimismo se observó la acumulación de grandes cantidades de proteínas acuosolubles ricas en prolina en las primeras etapas del desarrollo, que tienden a desaparecer con la edad. Se supone que actuarían como fuente de la conversión Pr-Hpr, previa a la síntesis del colágeno. Es registrada también una correlación entre la Hpr. hallada en estas complejas fracciones y la edad.

El descenso registrado en el nivel de prolina libre concuerda con los demás resultados obtenidos. La disminución de Hpr. libre, en cambio, es difícil de interpretar, pretendiéndose relacionarla con una teoría esbozada por Jackson para el granuloma.

Significado histoquímico da coloração da neuro-secreção pela hematoxilina crômica e pela aldeido-fucsina. W. A. Hadler e L. Costacurta. *(Depto. Morfologia, Fac. Medicina, Univ. São Paulo, Ribeirão Prêto, SP, Brasil).*

Admite-se que a coloração da neuro-secreção pela hematoxilina crômica e pela aldeido-fucsina seja conseqüência à elevada proporção de cisteina contida nos fatores hipofisários. A cisteina, sob a ação oxidante do permanganato, se transformaria em ácido cisteico, responsável pela coloração.

Todavia, com base na solubilidade da substância Gomori-positiva em solventes de lípides, aventamos a hipótese de ser essa substância de naturaleza lipídica.

Esta hipótese foi verificada de 2 maneiras:

a) lípides extraídos de neuro-hipófises de boi, por misturas etanol-éter a 1:1, foram colocados em papel de filtro sob a forma de mancha ("spot"), o qual foi corado pela hematoxilina de Gomori e pela aldeido-fucsina.

b) Soluções de diversas substâncias de naturaleza lipídica foram colocadas em papel de filtro, sob a forma de manchas, corados pelos mesmos corantes.

Os lípides extraídos da neuro-hipófise coraram-se nitidamente por ambos os corantes, apenas após oxidação prévia com permanganato-ácido sulfúrico.

Das diversas substâncias de naturaleza lipídica usadas (triglicérides, ácidos olêico, palmítico e esteárico, hidrocarbonetos parafínicos, colesterol, prednisona, desoxicorticosterona, benzoato de estradiol, calciferol e lecitina) apenas o colesterol, o calciferol e a lecitina se coraram, após oxidação permangânica. A lecitina usada continha colesterol como impureza (R. de Lichermann-Burchard positiva); por isso foi considerado o colesterol como responsável pela sua coloração.

Partindo dêsses resultados foram investigadas as eventuais transformações sofridas pelo colesterol e qual o produto final responsável pelas colorações da hematoxilina crômica e da aldeido-fucsina. Foi verificado: 1. o bloqueio pelo Br antes da oxidação inibe as colorações, o que revela a importância da dupla ligação; 2. o bloqueio pela anilina ou pelo 2.4-dinitrofenilhidrazina após a oxidação inibe as duas colorações; 3. o produto formado pela ação do permanganato sôbre o colesterol é redutor (reduz os sais de argentamina), mas é Schiff negativo. Os resultados do ítem 2 combinados com os do ítem 3 sugerem tratar-se de substância de naturaleza cetônica. 4. após oxida-

ção do colesterol pelo permanganato forma-se substância fortemente basófila e metacromática o azul de toluidina, em pH2, revelando tratar-se de produto ácido; 5. a metilação, feita após oxidação, inibe as duas colorações, o que mostra a importância dos grupos ácidos.

Desta forma seriam cetoácidos os responsáveis pelas colorações da hematoxilina crônica e da aldeído-fucsina, apresentada pelo colesterol após oxidação permangânica.

No que concerne à neuro-secreção, substâncias de naturaleza lipídica, dotadas de estrutura semelhante ou não à do colesterol, mas capazes de dar origen a cetoácidos após oxidação, seriam responsáveis pelas colorações em estudo.

Ação de algunos solventes de lípides sôbre a substância Gomori-positiva da neurohipófise. W. A. HADLER E A. R. CRUZ. *(Depto. Morfologia, Fac. Medicina, Univ. São Paulo, Ribeirão Prêto, SP, Brasil).*

Neuro-hipófises de ratos foram submetidas à ação de alguns solventes de lípides, antes, durante e após a fixação pelo ácido pícrico-formol. Foram usados como solventes: acetona, clorofórmio, etanol, etanol-éter 1:1, etanol contendo 1 % de ácido clorídrico e piridina. Além disso, soluções aquosas de ácido acético, nítrico e clorídrico a 5 % foram também empregadas.

Através de coloração pela hematoxilina crômica verificou-se que a substância Gomori positiva é totalmente extraída quando o material é tratado antes de fixação pelos seguintes solventes: etanol-éter, clorofórmio, etanol-ácido. A extração se efetua, mas bem menos intensa quando feita pelos demais solventes.

Quando a fixação é efetuada em solução de ácido pícrico a 1.2 % em etanol, acetona ou etanol-éter, verifica-se extração total da neuro-secreção. A adição de formol a 10 % às soluções de ácido pícrico impede a extração.

Após fixação pelo formol a 10 % nenhum dos solventes de lípides usados extraem a substância Gomori positiva.

As reações do ácido periódico-Schiff e da aloxana-Schiff, efetuadas em neuro-hipófises submetidas à extração pela mesma técnica, revelam que o material Gomori positivo, PAS positivo e aloxana-Schiff positivo, são diferentes entre si, uma vez que não se comportam da mesma maneira quando submetidas à ação dos solventes usados. Todos êsses três componentes da neuro-secreção são demonstráveis após fixação pelo bicromato de potássio-bicloreto de mercúrio e apresentariam naturezas diversas, quais sejam: a) substância solúvel nos solventes de lípides empregados e corável pela hematoxilina-crômica de Gomori: provável natureza lipídica; b) substância solúvel em soluções ácidas, parcialmente solúveis em etanol-éter e PAS positiva; provável polissacáride; c) substância solúvel em soluções ácidas e em água destilada e, aloxana-Schiff positiva: provável natureza protéica.

A ação do formol preservando a extração de substância Gomori pelos solventes de lípides sugere que a fração protêica deve estar ligada ao material lipídico, constituindo lipoproteína.

Ultraestructura y función de la glándula pineal. A. P. DE IRALDI. *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

El estudio con microscopio electrónico de la pineal de ratas blancas (1) muestra que está constituída por un solo tipo de células parenquimáticas, el *pinealocito*, con *expansiones claviformes plurivesiculares* unidas a las células por pedículos delgados de estructura túbulofibrilar.

Estas expansiones, situadas en amplios espacios pericapilares contienen abundantes vesículas de un diámetro promedio de 425 angstroms, unas homogéneas claras y otras provistas de un gránulo denso, en los preparados fijados con tetróxido de osmio, signo morfológico de la actividad secretoria de la glándula. Estas vesículas se encuentran también en menor número en las zonas marginales del citoplasma celular.

Se estudia la vinculación de estas vesículas con las denominadas aminas biógenas mediante la administración de fármacos (reserpina (2) iproniazida y pirogalol y precursores).

Se hacen consideraciones sobre su inervación.

(1) DE ROBERTIS E. y PELLEGRINO DE IRALDI, A. 1960 *(enviado para su publicación).*

(2) PELLEGRINO DE IRALDI A. y DE ROBERTIS, E. *Experiencia, Marzo de* 1961.

Indicaciones morfológicas de una relación metabólica directa entre células visuales y glia en la retina del sapo. A. LASANSKY. *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Las fibras de Müller de la retina son células gliales cuyas prolongaciones ocupan todos los espacios intercelulares constituyendo así una matriz en la cual las neuronas retinianas parecen estar incluídas. Como consecuencia de esta disposición, en la retina no existe un espacio extracelular apreciable (Wald, F. y De Robertis, E., datos a ser publicados), por lo cual los metabolitos que llegan a las neuronas retinianas probablemente deben hacerlo pasando a través de las células de Müller. Es entonces posible concebir que las fibras de Müller representan células "nurse" en analogía a lo hipotetizado en el caso de otras células gliales (Hyden, H. y Pigon, A., J. Neurochem., 1960, 6:57).

Por lo tanto, fueron realizadas observaciones a fin de investigar, al nivel del microscopio electrónico, la posible existencia de manifestaciones morfológicas de tal hipotética función de las células de Müller. Se halló que, en la retina del sapo, las células de Müller contienen en sus prolongaciones numerosas mitocondrias carentes de crestas, las cuales pueden a veces ponerse en contacto directo con las células visuales fundiendo su membrana externa con la membrana plasmática de las células de Müller. Este hallazgo es interpretado como sugiriendo una relación metabólica directa entre células visuales y de Müller. La posible naturaleza de esta relación será discutida.

El tejido conectivo como reservorio del "pool" extravascular de proteínas séricas. R. E. MANCINI, J. M. DELLACHA, O. DAVISON Y B. ÁLVAREZ. *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Univ. Buenos Aires, Buenos Aires, Argentina).*

Diferentes fracciones del plasma normal de rata (albúmina, globulinas, fibrinógeno) fueron marcados con colorantes fluorescentes. Se determinó la cantidad de colorante unido a cada fracción, verificándose además sus movilidades electroforéticas y el poder coagulante del fibrinógeno después de marcados. Ratas adultas fueron inyectadas por vía endovenosa con dichas fracciones a razón de 20 a 50 mg/100 g peso y sacrificadas entre 3 minutos y 10 días después. Muestras de sangre fueron tomadas para analizar el decaimiento de las proteínas marcadas en la circulación. Otro lote de animales fué inyectado con las mismas fracciones proteicas pero no marcadas, aplicándose luego a los tejidos de estos animales el test histoinmunológico, empleando anticuerpos fluorescentes contra dichas fracciones. En ambos tipos de experiencias los tejidos adecuadamente preparados fueron analizados con el microscopio de fluorescencia. Se observó: 1. las fracciones séricas marcadas decaen en la circulación después de inyectadas y la curva con ligeras diferencias para cada fracción muestra dos componentes, uno rápido de 6-7 h y otro lento de 3 días de vida media; 2. después de la inyección endovenosa las albúminas y globulinas marcadas pasan por difusión desde la luz de los vasos al tejido conectivo intersticial, estromal y capsular, así como a pericondrios, tendones, cápsulas articulares y membranas basales. La acumulación en estos tejidos fué máxima alrededor de las 12 horas, luego decayó progresivamente para desaparecer a las 24 h; 3. el fibrinógeno marcado fué observado en la luz de los vasos pero no mostró difusión al tejido conectivo: 4. las proteínas marcadas fueron también acumuladas por los macrófagos y en el riñón reabsorbidas por las células de los tubos contorneados proximales, luego de ser filtradas por el glomérulo renal. En estas dos estructuras la acumulación fué progresiva hasta el octavo día, para desaparecer alrededor del 12°; 5. los tests histoinmunológicos demostraron que la distribución de las fracciones séricas no marcadas es semejante a la observada cuando se inyectaron las proteínas marcadas; 6. el lote de control, con ratas inyectadas endovenosamente con soluciones puras de colorantes fluorescentes, mostró un decaimiento en la circulación entre 45 y 90 minutos y leve incorporación por los macrófagos y túbulo contorneado proximal renal.

Influence of age in the appearance of the epithelial cap in the glomeruli of the mouse kidney. N. D. MOLINA AND N. M. MONTORZI. *(Inst. de Histolo-*

gía y Embriología, Fac. de Ciencias Médicas, U.N.C., Mendoza, Argentina).

We have observed that in swiss male mice 6 months old, the parietal layer of the Bowman capsule presents an epithelial cap very similar to the epithelia of the proximal convoluted tubule of the kidney. The cap is present in all the males and in less than 10 % of females. Their cells are low piramidal, with growing height towards the center and with a free border differentiated as a brush border. This border shows a positive phosphatase alkaline reaction and a periodic acid Schiff reaction. We have observed that this structure incorporates trypan blue when is injected subcutaneously.

The study of 4 to 9 days old mice of both sexes, shows that the Bowman capsule does not present the above mentioned cap.

The relation between appearance of the cap and the beginning of puberty in these animals, is discussed.

Vascular changes in the retina of diabetic rats. I. T. L. MUSACCHIO, N. PALERMO AND R. R. RODRÍGUEZ. *(Inst. Fisiología, Fac. Medicina, Univ. La Plata, La Plata, Argentina).*

White rats from our Institute strain with severe or moderate alloxan diabetes were used throughout the experiment; 40 of these animals were forced-fed during 16 months with weekly determination of glucose in urine and glycemia measured once a month. Alloxan injected non-diabetic rats were used as controls.

Under ether anesthesia and after ligature of the vena cava and abdominal aorta, they were injected, in the thoracic aorta and the ventricle, with silver carbonate. Afterwards the eyes were removed, fixed, the retina dissected and studied under the microscope.

The most characteristic changes were dilation of the vessels, specially the arteries and loss of the muscular fibers, without hemorrhages.

Studies in 15 rats with 3 to 7 months of diabetes after subtotal pancreatectomy show a parallelism between the degree and duration of the disease and the changes observed in the retina.

Spermatogenesis pattern in guinea pig testis. R. NARBAITZ, W. BISHOP * AND F. SCHMIED. *(Inst. Anatomía General y Embriología, Fac. Medicina, Univ. Buenos Aires, Argentina).*

During an earlier work, studying auto-allergic lesions in guinea pig testis, we could not find in the literature a description of the normal pattern of spermatogenesis in this animal.

Material of normal and injected animals was embeded, sectioned and stained by several methods (Feulgen, P.A.S. and Himes and Moriber's stain). For denomination of cell types Clermont and Leblond's description of guinea pig spermiogenesis (1952) was followed.

Through the study of sections, we found in normal testes four types of tubular sections with a characteristic cell population which always included three cell generations. The constant presence of these four types, as well as the constancy of the relative frequency of them, depends of an also constant timing of spermatogenesis and any alteration of this timing must be reflected by an alteration of cell population of tubules.

For practical purposes it is not even necessary to analyse the total cell population: it will suffice to take notice of the spermatid types present in each tubular section, which normally are: Type I (27 %) cap-phase; Type II (11 %) pre-achrosome: Type III (14 %) early Golgi and achrosome: Type IV (48 %) late Golgi and mature.

The examination of our experimental material showed no alteration of spermatogenesic rhythm. Our simple scheme may be also suitable for analysis of the action of hormones, various drugs and special diets on guinea pig testis.

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Variaciones de la sustancia Gomori-positiva en el órgano subcomisural de la rata en deshidratación. E. D. PADIN Y E. RODRÍGUEZ. *(Inst. Histología y Embriología, Fac. Ciencias Médicas, Univ. Nac. Cuyo, Mendoza, Argentina).*

Se estudió el órgano subcomisural en ratas adultas en condiciones normales y en ratas

hermanas privadas de agua durante 8 días. Los cerebros se fijaron por perfusión en Bouin diluído y los cortes sagitales se colorearon con la técnica de la fucsina-paraldehido de Gomori.

El epitelio del tercer ventrículo, en la zona conocida como órgano subcomisural presentó, en los animales normales testigos, gránulos Gomori-positivos alrededor de los núcleos y en el borde libre de las células en cantidad moderada y una tinción difusa en la zona intermedia. En las ratas deshidratadas se observó un notable aumento de los gránulos que se encuentran en el borde libre del epitelio y una reacción mas intensa en la zona intermedia, antes citada.

Estudio histoinmunológico del desarrollo del colágeno. M. Paz, B. Álvarez y O. Vilar. *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Con la idea de localizar al nivel celular y extracelular a las proteínas colágenas y/o sus precursores durante el desarrollo del tejido conectivo, colágeno total de tendón de pollo (soluble en ácido acético) y sus diferentes fracciones (neutro, citro y acuo soluble) fueron químicamente caracterizadas e inyectadas a conejos a fin de obtener anticuerpos heterólogos. El suero de estos animales fué analizado electroforéticamente y la presencia de anticuerpos explorada mediante fijación de complemento y test de Boyden. La fracción gama-globulina fué separada, marcada con colorantes fluorescentes y la reacción histoinmunológica realizada "in vitro" contra cortes de congelación de tejido conectivo de embriones de pollo, pollos recién nacidos y adultos. Como control, fueron hechas incubaciones similares con globulinas normales, reacciones de bloqueo utilizando globulinas anticolágenas no marcadas y adsorción del anticuerpo "in vitro" mediante contacto del antisuero con el antígeno correspondiente. Se observó 1. constitución química parecida en las diferentes fracciones del colágeno, 2. capacidad antigénica leve, revelada por el bajo título de los anticuerpos obtenidos, 3. localización de las proteínas colágenas antigénicas predominantemente en la substancia fibrilar intercelular en el conectivo juvenil y adulto y en cambio acentuadamente celular en los fibroblastos del conectivo embrionario, 4. diferencias antigénicas en las proteínas colágenas del tejido conectivo de diferentes órganos, 5. capacidad del método histoinmunológico para detectar en los fibroblastos a los precursores de las proteínas colágenas.

Captación del radiofósforo por la corteza cerebral aislada del gato. L. Pérez-Achard, E. García-Austt y M. A. Patetta-Queirolo. *(Instituto Neurología, Montevideo, Uruguay).*

En once gatos se estudió la captación del fosfato radiactivo marcado con P32 por la corteza cerebral aislada y normal. En tres gatos se estudió la captación de la seroglobulina marcada con I^{131} (IHSA).

Los resultados obtenidos fueron los siguientes:

1. La captación del P32 fué mayor en áreas totalmente aisladas que en las restantes regiones de la corteza cerebral.

2. La captación del P32 fué mayor en el centro del área cortical aislada que en su periferia.

3. La concentración del P32 fué mayor en toda la corteza del hemisferio cerebral donde se aisló un área, que en el opuesto y tanto más intensa cuanto más próxima a dicha área aislada.

4. El aislamiento cortical parcial y la simple manipulación de las envolturas encefálicas provocan un aumento de la captación del P32, pero de menor cantidad que en el caso de aislamiento total (cortical y subcortical).

5. El estudio de la captación de la seroglobulina marcada no aportó datos de significación.

Se vincula este aumento de la captación del P32 al mayor intercambio del fosfato de los ésteres fosfóricos, como consecuencia del mayor metabolismo del área aislada, debido a la actividad epiléptica incesante, presente en la misma.

The action of ribonuclease on onion root-tip cells. An electron microscope study. I. Pérez del Cerro. *(Inst. Ana-*

tomía General y Embriología, Fac. Medicina, Buenos Aires, Argentina).

Pancreatic ribonuclease, a protein of a molecular weight of about 15.000 is known to penetrate into the radicular cells producing mitotic anomalies, inhibition of growth and protein synthesis and a loss of basophilic components (RNA). In order to determine changes at the submicroscopic level which may correlate with these alterations, an electron microscope study has been made on onion root-tip cells, which had been immersed in a ribonuclease solution containing 5 mg per ml of enzyme (Sigma, crystalline) and for a period of 8 hours.

The results showed that only minor changes in the ultrastructure of the cell were seen. Ribosomes and nucleoli, where the bulk of the RNA of the cell are localized, appeared unmodified at the magnifications we employed even when its basophilia, as observed with the light microscope, was abolished. The membranous components of the ergastoplasm appeared notably increased; in some cells these membranes fold forming concentric complexes. Occasionally we also observed large homogeneous structureless cytoplasmic zones where the organoids have been pushed to its periphery. No alterations have been seen in other cell structures (chromosomes, mitochondria, dictiosomes, etc.).

It would be interesting to investigate further this apparent disagreement between electron microscope and cytochemical data by studying the RNA of subcellular particles isolated from ribonuclease-treated cells in order to obtain a better understanding of the interesting action of this enzyme on living cells.

Histología normal de la mucosa del esófago y estómago del *Bufo arenarum* Hensel. Z. Riviello y S. Melamed. *(Inst. Histología y Embriología, Fac. Ciencias Médicas, Univ. Nac. Cuyo, Mendoza, Argentina).*

Como en otros anfibios, se observan en el esófago, glándulas túbuloalveolares ramificadas con dos clases de células, la mayoría mucosas y algunas zimogénicas. El epitelio es seudoestratificado ciliado con células caliciformes y la muscularis mucosa está muy pobremente definida.

La porción siguiente, estómago anterior, presenta un epitelio superficial simple columnar con algunas células ciliadas y caliciformes y una muscularis mucosa bien definida. Las glándulas más numerosas son del tipo tubular simple o ramificado, con los mismos tipos celulares del esófago aunque predominando las zimogénicas.

En la región fúndica, a diferencia de otros anfibios, se reconocen dos zonas por las características celulares de las glándulas, la inmediata al estómago anterior presenta un predominio franco de células zimogénicas, mientras que la segunda zona se distingue, por tener los cuerpos y fondos glandulares constituídos en su mayoría por células parietales. Las células mucosas del cuello se disponen en ambas zonas en la porción alta de las glándulas. El epitelio superficial es columnar simple mucosecretante y las glándulas de tipo tubular simple son las más largas del estómago.

La zona intermedia se caracteriza por presentar glándulas más cortas y con las células mucosas del cuello dispersas a lo largo del cuerpo y fondo glandular, interpuestas entre las células parietales.

La zona pilórica presenta glándulas tubulosas ramificadas más cortas con predominio de células semejantes a las del cuello. La muscularis mucosa en las regiones fúndica y pilórica alcanza el máximo desarrollo.

Correlación química y estructural en el cerebro — a) Aislamiento de terminaciones nerviosas en el cerebro de la rata. E. de Robertis, A. P. de Iraldi, G. Rodríguez de Lores Arnaiz y L. Salganicoff. *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Con el objeto de establecer una posible correlación entre las vesículas sinápticas y los mediadores químicos de la transmisión (1, 2) se han aislado diversas fracciones subcelulares del cerebro de rata por centrifugación diferencial. En vista de los resultados previamente obtenidos (3) se trató de aislar terminales sinápticos intactos en lugar de vesículas sinápticas. Luego de la homogeneización se centrifugó a 900 g para el aislamiento de los núcleos y a 11500 g para la fracción mito-

condrial. La fracción mitocondrial fué colocada en un gradiente discontinuo de sacarosa con cuatro niveles: 0.8; 1.0; 1.2 y 1.4 M.

Se obtuvieron cinco fracciones que se estudiaron con el microscopio electrónico, las cuales mostraron los siguientes componentes:

1. Fracción A (0.8 M, parte superior) constituída esencialmente por fragmentos de mielina.

2. Fracción B (0.8 M, parte inferior) heterogénea, formada por grandes vesículas que contienen vesículas sinápticas, fragmentos de membranas, cuerpos densos alargados, estructuras similares a los componentes subsinápticas (4) y escasas láminas de mielina.

3. Fracción C (entre 1.0 y 1.2 M) constituída casi exclusivamente por terminaciones sinápticas grandes y fragmentos de las mismas y algunas membranas y pequeños corpúsculos no identificados.

4. Fracción D (entre 1.2 y 1.4 M) correspondiente a terminaciones nerviosas de diferente tamaño, con su contenido en vesículas y mitocondrias.

5. Fracción E, sedimento compacto, constituído casi exclusivamente por mitocondrias.

(1) De Robertis, E. and Bennett, H. S.: *Fed. Proc.*, 1954, *13*, 35.

(2) De Robertis, E. and Bennett, H. S.: *J. Biophys. Biochem. Cytol.*, 1955, *1*, 47.

(3) De Robertis, E., Pellegrino de Iraldi, A., Rodríguez, G. and Gómez, C. J.: *En Prensa.*

(4) De Roberti:, E., Pellegrino de Iraldi, A., Rodríguez de Lores, Arnaiz, G. and Salganicoff, L.:*Anat. Rec.*, Febrero de 1961.

Probable mecanismo de formación de los cuerpos de Herring hipotalámicos.

E. Rodríguez y M. E. Gómez. *(Inst. Histología y Embriología, Fac. Ciencias Médicas, Univ. Nac. Cuyo, Mendoza, Argentina).*

Las presentes observaciones se realizaron en el hipotálamo de sapos *Bufo arenarum Hensel* mantenidos en inanición durante tres meses, ya que de acuerdo con observaciones anteriores los cuerpos de Herring aumentan en cantidad y tamaño en esas condiciones. El material se extrajo en distintos momentos del período de inanición, se fijó en Bouin diluído y se estudió en cortes frontales coloreados con la fucsina paraldehida de Gomori.

Dos tipos de cuerpos de Herring se distinguen por su ubicación: a. los encontrados en el tercio inferior de la pared del tercer ventrículo y b. los observados en el trayecto de los axones perpendiculares al tercer ventrículo.

El sacrificio gradual de los sapos en inanición mostró el predominio de ciertas imágenes que se interpretaron como etapas progresivas en la formación de los cuerpos de Herring. Las etapas iniciales estarían representadas por aquellos axones que presentan pequeños gránulos del material Gomori-positivo en forma de un rosario. Le siguen dilataciones vesiculares del axón que contienen varios gránulos en su interior.

La fusión de dichos gránulos formaría conglomerados densos y homogéneos en el trayecto del axón de tamaño mucho mayor que los gránulos del rosario. Estos conglomerados pueden aparecer en distintos puntos del mismo axón o sea que cada axón podría tener más de un cuerpo de Herring.

Correlación química y estructural en el cerebro — b) Distribución de la acetilcolinesterasa en fracciones subcelulares de cerebro de rata.

G. Rodríguez de Lores Arnaiz. *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

En las distintas fracciones celulares del cerebro de rata preparadas según a), se determinaron las actividades de acetilcolinesterasa y de succinodehidrogenasa refiriéndose los resultados a la distribución de proteínas.

El sedimento de 900 g mostró baja actividad de acetilcolinesterasa y de succinodehidrogenasa. El sedimento de 11500 g presentó aproximadamente la mitad de la acetilcolinesterasa del homogeneizado total y la casi totalidad de la actividad de la succinodehidrogenasa.

El sobrenadante de 11500 g tuvo una actividad de acetilcolinesterasa semejante a la fracción anterior, pero con baja actividad de succinodehidrogenasa.

En las subfracciones del gradiente se obtuvieron los resultados siguientes:

1. falta de acetilcolinesterasa y de succinodehidrogenasa.

2. alta actividad de acetilcolinesterasa y sin succinodehidrogenasa.

3. alta actividad de acetilcolinesterasa y baja actividad de succinodehidrogenasa.

4. baja actividad de acetilcolinesterasa y mayor actividad de succinodehidrogenasa que en c).

5. baja actividad de acetilcolinesterasa y alta actividad de succinodehidrogenasa.

Estos resultados serán discutidos en relación con la posible localización funcional de la acetilcolinesterasa en la región sináptica y el problema de la diferenciación entre sinapsis colinérgicas y no colinérgicas.

Correlación química y estructural en el cerebro — c) Distribución de la acetilcolina en fracciones subcelulares del cerebro de rata. L. SALGANICOFF. *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Se determinó la relación acetilcolina/proteína en cada una de las fracciones del cerebro de rata y en las subfracciones del gradiente descriptas anteriormente.

La fracción A no contiene prácticamente acetilcolina siendo su coeficiente de actividad específica nulo.

La fracción B contiene acetilcolina en proporciones variables siendo su coeficiente de actividad específica relativamente alto.

La fracción C es la que tiene mayor cantidad de acetilcolina en relación a proteínas.

La fracción D tiene acetilcolina, pero su cantidad específica es mucho menor que C.

La fracción E no contiene acetilcolina.

Estos resultados unidos a los mencionados en los trabajos anteriores demuestran la existencia de una población diferente de terminaciones nerviosas en las diversas subfracciones. La fracción C correspondería preferentemente a terminaciones colinérgicas y la fracción D a sinapsis no colinérgicas.

Leucine-H^3 incorporation into the exocrine pancreas of the growing rat. A. SESSO AND J. CARNEIRO. *(Depto. Morfología, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil; Labor. Fisiología Celular, Fac. Medicina, São Paulo, Brasil).*

Five μc/g of body weight of leucine-H^3 were injected into rats aged 2, 4, 10, 15, 20, 33, and 40 days. From each, animal biopsies were taken at 15 minutes and 4 hours after the injection; 8 hours after the last biopsies, the animals were sacrificed and the remainder of the pancreas taken for examination.

Radioautographs were made by dipping the microscopic slides into melted NTB3 emulsion, according to the technique of Messier and Leblond (1957). The preparations were exposed during 20 days.

Fifteen minutes after the injection of the labeled amino acid the following observations were made: the animals 10 days old or younger showed no incorporation in the exocrine pancreas. After the 15th day of age, the acinar cells showed an incorporation, which was more pronounced in the older rats.

The biopsies taken 4 hours after the injection, showed incorporation of the amino acid in all animals. However, this incorporation was weak in the 2, 4, and 10 days old rats, and intense in the ones 33 and 40 days old. In the last animals the silver grains were located over the apical pole and the upper third of the acinar cells.

Twelve hours after the leucine-H^3 injection, the animals aged 2, 4, and 10 days showed a picture indicating an accumulation of the label in the upper third and apical pole of the cells. In the animals with 33 and 40 days of age, the label was present mainly in the apical pole of the acinar cells and into the lumens of the acini.

The observations described indicate that just after delivery, the acinar pancreatic cells exhibit a weak capacity of synthesis and extrusion of digestive enzymes. After the 15th day of age, the velocity of synthesis and extrusion increase progressively.

Effects of thyroxine and cortisone on the amylase and trypsin activable protease activities of the pancreas and pancreatic juice of the hypophysetomized rat. A. SESSO AND V. VALERI. *(Depart. Morphology, Faculty of Medicine of Ribeirão Prêto, São Paulo, Brasil).*

Female rats, hypophysectomized 30 days before, were given dl-thyroxine (Tx) (4 μg/100g/day during 6 days) and cortisone acetate (Cort). Cort was used in two doses (80 and 150 μg/100g/day during 6 days). After

11 hours of fasting the pancreatic juice was withdrawed for recording of the hourly flow and also for assay the amylase and trypsin activable protease activities (TAPase). In the homogenates concomitantly with enzymatic assay, biochemical determinations of nucleic acid were carried out. The DNA of the acinar cells was measured histophotometrically. No changes in the mean DNA contents of the diploid nuclei of the various experimental groups could be detected.

Tx raises the extrusion, per unit time and per cell, of amylase and TAPase (amylase/hour/DNA and TAPase/hour/DNA) into the juice. Cort, in the two levels used, raises only the extrusion of amylase per hour and per cell; the extrusion of TAPase in the hypophysectomized rats injected with Cort is not appreciably modified.

In the homogenates from the animals injected with Tx, a raise in the amylase activity per cell (amylase/DNA) occurs, while no modifications are detectable in the TAPase activity per cell (TAPase/DNA). Cort in the 80 μg doses raises only the amylase activity per cell in the homogenates. In the doses of 150 μg, Cort raises both, the amylase and TAPase contents of the acinar cells.

Studying the relation: enzyme discharged per hour into the juice/enzyme contents of the gland, the following was observed:

1 — Tx raises the relation for both, amylase and TAPase.

2 — Cort in the 80 μg dose does not appreciably modifies it.

3 — Cort in the 150 μg dose diminishes it for both amylase and TAPase.

The RNA content per cell (RNA/DNA) increases in the cells of the hypophysectomized animals injected with Tx and Cort in the dose of 150 μg. In the hypophysectomized rats injected with Cort in the 80 μg level, no changes in the RNA/DNA relation could be detected.

Assuming, as we did, that enzyme activity in the above conditions is proportional to the actual enzyme content of the samples, the data herein presented indicate that the velocites of synthesis and extrusion of amylase and TAPase are differently affected by Tx and Cort. Furthermore it seems that Tx acts mainly by favouring the extrusion of digestive enzymes while Cort, according to the dose, enhances the accumulation of zymogen material in the cytoplasm of the acinar cells.

Manifestaciones citológicas y citoquímicas del envejecimiento en las neuronas ganglionares raquídeas. J. M. Sosa. *(Inst. Histología, Fac. Medicina, Univ. Los Andes, Mérida, Venezuela).*

Se describen los resultados de investigaciones morfológicas y citoquímicas en las neuronas ganglionares raquídeas del conejo, durante el ciclo vital y en relación con el proceso de envejecimiento. Las investigaciones se refieren a: 1. la forma y citoquímica de las neuronas redondas y esquinadas; 2. las formas yuxta y perinuclear del aparato de Golgi; 3. los distintos grados de integridad del aparato de Golgi, que se clasifican en seis etapas o estadios diferentes. Los períodos en que recaen las investigaciones son: el fetal, el nacimiento, el primer mes de vida extrauterina, el primer año y el tercer año.

He aquí los resultados:

1. Los distintos aspectos morfológicos del aparato reticular de Golgi (estadios I-VI) son variaciones correlativas a estados funcionales;

2. Estas variaciones funcionales aparecen durante el ciclo vital, según un plan y ritmo gráficamente representables;

3. La fragmentación del aparato reticular se acentúa con la diferenciación, con el grado de actividad y con el envejecimiento (envejecimiento celular y envejecimiento del animal);

4. La forma yuxtanuclear del aparato reticular de Golgi es más propia de las neuronas ganglionares jóvenes o en proceso de desarrollo, mientras que la forma perinuclear es representativa de estadios más maduros o plenamente desarrollados;

5. Las células esquinadas representan, tal como intuyó Ramón y Cajal, neuronas decadentes, desgastadas, es decir, etapas finales o prefinales del ciclo vital de las neuronas ganglionares raquídeas, neuronas envejecidas.

Reconstrucción tridimensional de las vías excretoras del esperma en el hamster (rete testis, conductos eferentes y epididimo). A. O. Stati y N. M. Montorzi. *(Inst. Histología y Embriología, Fac. Ciencias Médicas, Univ. Nac. Cuyo, Mendoza, Argentina).*

En material fijado en formol buffer e incluído en parafina, se realizaron cortes seria-

dos de 10 micrones que se proyectaron y dibujaron para la reconstrucción.

La rete testis se dispone a nivel del polo vascular del testículo formando un casquete de sacos aplanados, irregulares e intercomunicantes. Los túbulos seminíferos se continúan por túbulos cortos rectos que presentan válvulas epiteliales al desembocar en la rete testis.

La confluencia de los sacos en la parte central de la rete testis, se prolonga en forma de infundíbulo hacia los conductos eferentes y tiene un trayecto fuera del testículo de aproximadamente 0.8 mm. A este nivel presenta tabicamientos que sirven de punto de partida a los conductos eferentes en número de 7 a 9.

Los conductos eferentes tienen un trayecto rectilíneo en la porción inferior y se hacen muy tortuosos en la superior. Al llegar a la cabeza del epidídimo confluyen reduciéndose en número, para formar un túbulo único, sumamente tortuoso en la primera porción que pasa a la segunda porción con todas las características del epidídimo.

Se describen las características epiteliales de las diferentes zonas.

Observações sôbre os mastócitos humanos. L. C. UCHÔA-JUNQUEIRA E N. FAUSTO. *(Lab. Fisiología Celular, Fac. Medicina São Paulo, Univ. São Paulo, Brasil).*

Contribution to the study of leukocytes migration. CLARA E. R. DE VARGAS AND M. H. BURGOS. *(Inst. de Histología y Embriología, Fac. de Ciencias Médicas, U.N.A.C., Mendoza, Argentina).*

The study of the structure of the vaginal mucosa during the sexual cycle in the hamster as in other rodents, shows, during metestrus, numerous leukocytes (mostly neutrophilic) leaving the blood vessels and going accross the vaginal epithelium toward the vaginal lumen. This migration which starts a few hours after ovulation, was studied with the electron microscope in material fixed in Palade fixative with sucrose and embedded in prepolymerized methacrylate.

Leukocytes cross the capillary endothelial wall apparently through the intercellular spaces by cytoplasmic prolongations which separate desmosomes. When the leukocytes enter into the intercellular space between the vaginal epithelial cells, they compress these epithelial cells making the microvilli disappear in the points of contact.

When the leukocytes on their way towards the vaginal surface leave the intercellular space, the epithelial cells recover their original shape, the desmosomes become attached again and the microvilli reappear.

Datos cuantitativos de la variación con la edad, del tamaño de los folículos de la tiroides de la rata blanca. E. A. DA VIÁ. *(Inst. Histología y Embriología, Fac. Ciencias Médicas, Univ. Nac. Cuyo, Mendoza, Argentina).*

El estudio se realizó en dos grupos de animales: 1. animales de uno a cuatro días de edad; 2. animales de un mes. Para la fijación, inclusión y coloración se utilizaron técnicas de rutina. En cada preparado se midieron los diámetros de los folículos y la altura de sus epitelios, utilizándose doce animales y un total de más de 800 determinaciones.

El tratamiento estadístico de estos resultados revela, al comparar los folículos periféricos y los centrales del primer grupo, que no existen diferencias significativas en los diámetros de dichos folículos ni en la altura de sus respectivos epitelios; determinándose los valores término medio de 32 micrones para el tamaño de los folículos y 6.2 micrones la altura de los epitelios.

El mismo tratamiento aplicado al segundo grupo, revela notables diferencias entre los diámetros de los folículos centrales y periféricos. El término medio de las determinaciones realizadas es de 75 micrones para los periféricos y 31 micrones para los centrales. La altura de los epitelios revela también diferencias significativas, siendo de 4 micrones para los folículos periféricos y de 6.3 micrones para los centrales.

Estos resultados indicarían que la característica diferencia entre los folículos periféricos y los centrales de la tiroides de la rata blanca, se produciría durante el primer mes de vida, estando ausente hasta los primeros cuatro días de nacer.

Participación del riñón en la incorporación de proteínas séricas y hormonas proteicas marcadas. O. VILAR, J. M. DELLACHA, O. W. DAVIDSON Y B. ÁLVAREZ. *(Inst. Anatomía General y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Proteínas séricas de rata y diversas hormonas pituitarias (Armour y NIH, USA) fueron marcadas con colorantes fluorescentes. Dichas proteínas fueron controladas antes y después del marcaje por electroforesis y pruebas de actividad biológica. Por vía intravenosa fueron luego inyectadas a ratas en dosis variables de 1 a 50 mg/100 g de peso y sacrificadas a intervalos variables, entre 3 minutos y 10 días después. Muestras de sangre y de orina fueron tomadas para medir el decaimiento de dichas proteínas en la circulación y detectar su posible eliminación por riñón. Este órgano fué finalmente adecuadamente fijado y preparado para su análisis con el microscopio fluorescente. Como control otros animales fueron inyectados con soluciones puras de los colorantes fluorescentes utilizados y sometidos al mismo esquema de análisis.

Se observó que el decaimiento en la circulación de dichas proteínas es acompañado de su eliminación a través del glomérulo renal y consecutiva reabsorción predominante por el túbulo contorneado proximal. Pequeñas cantidades de algunas de estas proteínas pueden pasar a los túbulos restantes y ser reabsorbidas en el asa de Henle, tubo contorneado distal y aún en los colectores o bien aparecer en la luz de la vejiga y en la orina. Cuando se inyecta el colorante fluorescente libre, puede aparecer acumulado en pequeña cantidad únicamente en el túbulo contorneado proximal.

Las diferencias observadas durante este proceso entre las proteínas séricas y las hormonas proteicas permiten interpretar que esta función del túbulo renal estaría en relación con la naturaleza de la proteína, su peso molecular y/o las dosis administradas.

Distribution of labelled serum proteins within the renal vessels during diuresis and antidiuresis. M. F. VILLAMIL. *(Inst. Anatomía General y Embriología, Univ. Buenos Aires, Buenos Aires, Argentina).*

It has been recently demonstrated that the concentration of solute in different structures of the renal parenchyma increases from the cortex towards the tip of the papilla. Data concerning proteins are scarce and contradictory: according to radioautographies the protein concentration increases in the papilla but micropunctures of the vasa recta were unable to confirm this finding.

Two groups of rats were injected with homologus serum labelled with lisamine rhodamine P200 and killed 15 hours later, the kidneys being examined under the fluorescent-microscope. One group was deprived of water during 24 to 48 hours while the other received during three hours previous to being sacrificed an intravenous infusion of 25 % mannitol at a rate of 0.17 ml/min, a profuse diuresis of 20 to 40 ml being thus obtained.

In the dehydrated animals accumulation of labelled proteins within the vasa recta of the medulla was found. Osmotic diuresis promoted by mannitol either attenuated or wholly prevented this accumulation.

It is concluded that the plasma proteins within the vasa recta participate in the countercurrent mechanism which is counteracted by osmotic diuresis.

Contribución al estudio del epitálamo en el *Bufo arenarum* Hensel. R. O. VITALE. *(Inst. Histología y Embriología, Fac. Ciencias Médicas, Univ. Nac. Cuyo, Mendoza, Argentina).*

El epitálamo de sapos adultos de ambos sexos, *Bufo arenarum Hensel* presenta en la vecindad de la zona pineal, dos formaciones separadas por la comisura de la habénula, una piramidal con vértice hacia el tercer ventrículo: la paráfisis cerebral y otra posterior de forma ovoide alargada: la epífisis.

La epífisis presenta dos folículos, uno anterior grande y otro posterior de menor tamaño, ambos tapizados por un epitelio simple cuboide. Tres tipos celulares se distinguen en este epitelio: 1. cuboideo sin prolongaciones, que son mayoría; 2. cuboide con prolongaciones citoplasmáticas y 3. columnar delgado o triangular, probablemente de sostén. Estos

folículos están rodeados por tejido conectivo rico en capilares en donde se observan algunas células cebadas.

La paráfisis ubicada por delante de la epífisis, presenta también folículos tapizados por un epitelio simple cuboideo. En algunos animales en la parte superior se destaca un folículo grande e irregular, estando la inferior ocupada por varios pequeños; en otros predominan en ambas partes los folículos pequeños. En ocasiones se ve alguno de estos folículos comunicar con el tercer ventrículo.

Rodean a los folículos tejido conectivo laxo y numerosos vasos sanguíneos. La capa de melanóforos que acompaña a las meninges contacta con la base de la paráfisis.

Se discute y se hacen resaltar las características especiales del epitelio del epéndimo en las vecindades de la epífisis y antes de llegar al órgano subcomisural.

Active transport of sodium in the isolated frog brain. J. A. ZADUNAISKY AND P. F. CURRAN. *(Inst. of Biological Chemistry, Univ. of Copenhagen and Cát. de Física Biológica, Fac. de Ciencias Médicas, Buenos Aires, Argentina).*

The brain of the european frog *Rana temporaria* when placed *in vitro* shows no appreciable weight change, in contrast with the mammalian brain. This property has been useful for the study of fluxes with radioactive Na. The results were treated assuming the presence of three compartments of constant volume.

The efflux curves consist of two exponentials. On the basis of the first and more rapid, the diffusion coefficient of Na was calculated and the result was near to the theoretical one for diffusion of Na in water. The calculation of the volume of distribution of this fast Na fraction was similar to the inulin "spaces" obtained in this brain preparation. This indicates that there is experimentally a compartment with the characteristics of an "extracellular space".

The second and slower component, is affected by factors that are known to inhibit active Na transport. Metabolic inhibitors like Na iodoacetate, KCN, ouabain and low temperature, reduce the slope of Na 24 efflux.

The values for Influx and Efflux are coincident and agree with the constancy of the total stable Na content.

The slow component is interpreted as an active Na transport and the first one, faster, as the diffusion of Na from a high Na containing compartment which behaves as an extracellular space.

Osmotic changes and ionic equilibria in isolated frog brain. Function of glial cells. J. A. ZADUNAISKY, F. WALD AND E. D. P. DE ROBERTIS. *(Cát. Física Biológica e Inst. Anatomía Normal y Embriología, Fac. Ciencias Médicas, Buenos Aires, Argentina).*

Observation of the central nervous system with the electron microscope does not show the presence of extracellular space. When mammalian brain slices are incubated in hypotonic fluids there is a gain of water localized in astroglia (Gerschenfeld et al., 1959).

The brain of the south american frog *Leptodactylus ocellatus*, when placed in hypotonic or hypertonic media shows modifications of volume which deviate from the Boyle-Van't Hoff relation, indicating a restrain to osmotic changes. In this case there is a volume change in the ependimal glial cells which correspond to the astroglia of mammals. The other elements of the central nervous system show little or no change. Penetration of KCl, when part of the NaCl of the Ringer is replaced with the K salt, produces, as in other tissues, a simultaneous gain of water. The observation with the electron microscope reveals an immediate swelling of the glial processes and later on a clarification of the endoplasmic reticulum of the neurons. This is interpreted as a primitive entrance of KCl and water into the glial cells and secondarily into the neurons.

The cardiotonic glucoside ouabaine, produces a reduction in Na 24 efflux from these brains and simultaneously an increase in water content.

Under these conditions, there is a swelling of the glial processes, with little reaction in the rest of the elements of the C.N.S. The increase in water content due to retention of Na, accounts for the reduction in active Na transport.

These results are interpreted as follows: the glial compartment would be in osmotic contact

with the external fluid but not the rest of the elements of the central nervous system, the glia also being in osmotic relation with the neurons.

NEOPLASIAS
NEOPLASIES

Hiperplasia y tumores de mama inducidos por la yodotiroidectomía. A. F. CARDEZA, R. NALLAR Y E. A. PORTA. *(Inst. Biología Medicina Experimental, Buenos Aires, Argentina).*

Se realizó la tiroidectomia administrando 1 μc de I^{131} a ratas blancas hembras enteras de 200 a 250 gramos de peso corporal. El ciclo vaginal se controló la primera y tercera semana de cada mes. Los animales se sacrificaron por grupos, en tiempos diferentes que fueron desde 10 días hasta 14 meses.

En todas las ratas se observó el espaciamiento cada vez mayor de los estros, concordando este fenómeno con la aparición de los signos de hipotiroidismo, hasta la desaparición total de los ciclos estrales en la mayoría de los animales.

Histológicamente los cambios morfológicos fueron:

1. Las hipófisis mostraron en los primeros meses, abundantes células basófilas centrales hialinas y disminución de acidófilas. Posteriormente hubo disminución de basófilas y aumento correlativo de acidófilas y cromófobas. En 3 ratas sacrificadas después de los 12 meses se encontraron adenomas de células basófilas atípicas.

2. En glándula mamaria se observó hiperplasia túbulo-alveolar de grado intenso, con secreción láctea variable en el 50 % de las ratas entre los 9 y 12 meses después de la iodotiroidectomía. Dos animales presentaron fibroadenomas de tamaño variable entre un huevo de gallina y una mandarina.

3. En los ovarios de todos los animales hubo un predominio de cuerpos amarillos de gran tamaño.

4. En la vagina se observó intensa mucificación del epitelio, con cornificación simultánea de menor grado.

5. Las glándulas suprarrenales mostraron en los primeros meses un ensanchamiento de la cortical a expensas de la capa fasciculada. Más adelante presentaron focos de degeneración quística cortical. A los 12 meses había disminución de tamaño de la glándula a expensas de la cortical.

Tolerance of tissue homografts induced in adult mice by parabiosis *. C. MARTÍNEZ ** AND F. SHAPIRO. *(Depart. Physiology, Univ. Minnesota, Medical School, Minneapolis 14, Minn., U.S.A.).*

The method of parabiosis, performed between members of two inbred strains of mice or between a parent and its F_1 hybrid, was efficient in inducing acquired tolerance of skin homografts.

Adult mice of the Z (C3H) strain were joined in parabiosis with animals of the Ce strain or with F_1 obtained by crossing Z strain animals with A, C57B1/1 and Ce mice. Parabiosis was maintained for 20 to 60 days, after which the parabionts were separated. Eight days later the Z member of the pair was grafted either with Ce or with the corresponding hybrid skin. The percentage of Z mice becoming tolerant of the skin homograft varied with the different combinations, as follows: 100 % (10/10) in the Ce↔Z group; 100 % (26/26) in the (Z x Ce) F_1↔Z; 92 % (12/13) in the (A x Z) F_1↔Z and 20 % (2/10) in the (Z x C57B1/1) F_1↔Z group.

Induction of tolerance by means of parabiosis might be explained as a result of a continuous exchange of transplantation antigens in the form of intact cells between both members of the pair. These antigens in turn, presumably circulate and are exchanged in sufficient amount between the parabionts as to inhibit the capacity of the grafted animal to react against the homologous graft.

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** American Cancer Society Profesor of Physiology.

Leukemia induced by Sarcoma 180. CHRISTINNE D. DE PASQUALINI, E. A. D. HOLMBERG, S. L. RABASA AND A. PAVLOVSKY. *(Sec. Leucemia Experimental,*

Acad. Nac. Medicina, Buenos Aires, Argentina).

Rockland mice were immunized to Sarcoma 180 and it was observed that at death, 34 % of them developed leukemia. Moreover, 5 out of 21 of these animals presented a disseminated sarcoma similar to the original one. Both this leukemia and the generalized sarcoma originated in the carrier, since unlike Sarcoma 180 are not transpantable to any other strain. Attempts to transplant these leukemias in the original strain were successful in 3 cases, one of which is now in its thirteenth cellular passage (L - 180).

Mice immunized to sarcoma 180 are also immune to leukemia 180 an vice versa. This can be interpreted in the following way: the same subcellular agent would be responsible for the development both of sarcoma 180 and of leukemia 180 and as such responsible for the crossed immunity observed in these animals.

Lesiones gástricas del cobayo inducidas por inyección intramural de 3-metilcolantreno. R. S. DE ZALDÍVAR, C. ALIAGA Y H. RODRÍGUEZ. *(Cát. Biología, Esc. Medicina, Univ. Chile, Santiago, Chile).*

1. Cobayos de ambos sexos (14 machos y 11 hembras) recibieron una sola inyección de 0.05 ml de polietilen glicol-400 conteniendo 1 mg de 3-metilcolantreno, en la pared ventral del antro gástrico.

2. El experimento duró hasta 8 meses.

3. Lesiones gástricas benignas aparecieron en 14 de 25 animales (56.0 %). De estos 14 casos, 10 mostraron divertículos y 4 lesiones epiteliales infiltrativas. Estas lesiones aparecieron en la submucosa o en la musculatura del estómago. En 2 de estos 14 casos, se observó un área hiperplástica de la mucosa gástrica localizada sobre el sitio de la inyección.

4. Los animales con lesiones gástricas no mostraron diferencias con respecto al sexo.

Lesiones gástricas del cobayo inducidas por implante intramural de cristales de 3-metilcolantreno. R. S. DE ZALDÍVAR, C. ALIAGA Y H. RODRÍGUEZ. *(Cát. Biología, Esc. Medicina, Univ. Chile, Santiago, Chile).*

1. Cobayos de ambos sexos (21 machos y 20 hembras) fueron implantados en la pared ventral del antro gástrico con una dosis de 3 mg de cristales de 3-metilcolantreno.

2. El experimento se prolongó hasta 515 días.

3. Lesiones gástricas aparecieron en 14 de 41 animales (34.1 %). En 11 casos se observaron divertículos. Esta lesión se presentó en la submucosa o la *muscularis propria* y en la mayoría de los casos mostró aspecto normal de las glándulas y relaciones normales entre las células. En dos casos, la pared gástrica mostró focos de crecimiento epitelial atípico y en un caso, un leiomiosarcoma.

4. Los animales con lesiones gástricas no mostraron diferencias con respecto al sexo.

5. Hubo un caso con fibrosarcoma subcutáneo, localizado en el cuello del animal. El tumor destruyó casi toda la piel que lo cubría, pero no dió metástasis.

NEUROFISIOLOGÍA
NEUROPHYSIOLOGY

Fibrillation and sensitization in normally innervated skeletal muscle after tetanic stimulation. J. ÁLVAREZ. *(Labor. Neurofisiología, Univ. Católica, Santiago, Chile).*

Cats under penthobarbital anesthesia were used. The geniohyoideus neuromuscular preparation was utilized. After a tetanic stimulation (200/sec for 5 sec) applied to the nerve, the muscle showed: a) spontaneous electrical activity, made up of spike potentials (acute fibrillation), and b) a lowering of the threshold to acetylcholine. These phenomena lasted for a few minutes returning the muscle to its normal state. If stimulations were repeated at intervals of less than 30 minutes, these phenomena decreased or even disappeared. Neither curare nor prostigmin, injected during fibrillation modified its characteristics. Direct stimulation in a full curarized preparation was able to set up an acute fibrillation.

In this sense, the geniohyoideus muscle resembles the myotonic muscle, the denervated

muscle and muscles under the effect of some drugs (dichlorphenoxiacetic acid, veratrine, etc.). The existence of a common mechanism for all these conditions is suggested.

Influence of limbic system on blood pressure and respiration responses in the rat *. M. COVIAN, J. ANTUNES RODRIGUES AND V. MENDONÇA **. *(Depto. Fisiologia, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

The central nervous system plays an important rôle on the regulation of the neurovegetative functions, being the encephalic part the most important. Above the *medulla oblongata* the hypothalamus is the region that appears to be more related with cardiovascular control. The role played by the cerebral cortex in the regulation of the cardiovascular system is known since the first electrical stimulation made by Schiff, confirmed by Danileswsky, Bochefontaine and Richet. The limbic system is the other section of the encephale that in the last years has called the attention of many laboratories because of its influence on behaviour and neurovegetative functions. Previous studies have shown that stimulation of limbic structures of animals and man can produce several kinds of autonomic responses. In the present paper the cardiovascular and respiratory changes elicited by the electrical stimulation of different parts of the rat's limbic system are studied. Experiments were carried out on 76 Wistar male and female rats anesthetized with chloralose. The electrical stimuli were square waves of 10 msec duration, 40/sec, voltage variable, delivered by a Grass stimulator, during 10 seconds. The following structures of the limbic system were stimulated: orbital cortex of the frontal lobe; olfactory tubercle; pyriform cortex; amygdaloid complex; hippocampus; fornix; entorhinal area. The results obtained were as follows:

A fall in blood pressure was most frequently observed; only in two animals in which the medial nucleus of the amygdala was stimulated, a rise was elicited.

The respiratory response consisted, generally, in an increase of amplitude.

No consistent change in heart rate was observed.

The results reported seem to be mediated through the parasympathetic division of the autonomic nervous system; however the inhibition of the sympathetic division can not be disregarded.

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Interaction between the specific visual system and the reticular formation *. M. R. COVIAN AND R. F. MARSEILLAN. *(Depto. Fisiologia, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

Reticular stimulation has been shown to affect the sensory discharge at various levels in the central nervous system; the scientific literature regarding the physiological significance of the non-specific projection system and its interaction with the specific one has increased largely during the last years. The conclusions are that the reticular formation plays an important rôle in the integrative function of the brain, that is, in the integrative processes that organize states of consciousness. In a previous paper, Covian, Timo Iaria and Marseillan have shown that a single conditioning shock applied to the reticular formation preceding at a convenient interval the specific test stimulus (tactile) determined a reduction of the negative component of the evoked cortical potential and sometimes an increase of the positive wave. In an attempt to determine the effect of mesencephalic reticular formation stimulation upon the visual evoked potential in order to ascertain if there was a differential effect upon both components of the response, the present work was carried out.

The effect of the unspecific system upon the visual evoked cortical potential elicited by a flash light has been studied by single shock conditioning stimulation of the mesencephalic reticular formation at various intervals in cats under chloralose anesthesia. The conclusions arrived at during these experiments were the following:

There was a differential interaction of the postsynaptic components of the evoked response. An increase of the surface-positive, and

a decrease of the surface-negative phase were observed. This interaction seems to take place principally at cortical level.

The heterosynaptic convergence of the specific and unspecific systems in cortical neurons is given as a possible basis for the mechanism underlying this interaction.

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Efecto del ion Ca en concentraciones fisiológicas sobre el estado configuracional de las proteínas del nervio. M. LUXUORO-MARIANI. *(Instituto Fisiología, Univ. Chile, Santiago, Chile).*

Gold plated electrodes for permanent implantation. R. F. MARSEILLAN AND J. V. PEREIRA-LEITE. *(Depto. Fisiologia, Fac. Medicina, Ribeirão Prêto, São Paulo, Brasil).*

Electrodes for permanent implantation in the central nervous system should be made of inert metals to avoid chemical lesions. Stainless steel is usually preferred on those grounds; however, it is not available with enamel coating in all gauges. Besides, special techniques are needed for soldering.

Therefore, enamelled copper magnet wire electrodes were experimented, gold-plating the bare tips. A conventional electroplating bath of gold-potassium cyanide was used; one to three minutes of plating with a 1 mA current is generally sufficient for a 1mm² tip.

In order to test tissue tolerance to the electrodes, they were implanted in cats, along with stainless steel and bare copper electrodes of the same size. Impedance variation over time was measured and histologic studies undertaken. The lesions observed were slightly larger for the gold-plated than the stainless steel. The impedance of the gold plated electrode to a 1000 c/s signal was more stable over a three month period than that of the stainless steel or copper electrodes.

Interaction between visual and auditive evoked potentials. E. F. MIGLIARO, L. D'ANNA AND M. RIERA SÁNCHEZ. *(Lab. Fisiología, Fac. Medicina, Univ. de Carabobo, Valencia, Venezuela).*

Evoked potentials induced by sound and photic stimulation are obtained in chloralosed cats.

Potentials are recorded from both primary and "association" cortex with surface electrodes by superimposing of different sweeps from an oscilloscope, so eliminating the influence of any spontaneous variation.

In some conditions of stimulation, auditive and visual potentials modify each other, the final response presenting a complete different configuration to that of simple superimposing.

This modification is directly related to time delay. Thus an auditive stimulus applied 5 to 10 ms after a visual one, enlarges its amplitude and produces an acceleration in summit of its positive component. On the contrary, with 20 to 30 ms of delay between both stimuli, the visual potential presents a complex configuration without alteration of its initial component.

The modifications observed indicate mechanisms of facilitation or occlusion according to conditions of associating both stimuli.

Importancia de los factores periféricos en la habituación fótica cortical. M. PALLESTINI, V. ARMENGOL Y R. GALLARDO. *(Centro Psiquiatría Experimental, Univ. Chile, Santiago, Chile).*

En la habituación de los potenciales fóticos corticales se ha postulado una influencia inhibitoria que actuaría sobre la vía específica. En este trabajo estudiamos la importancia de algunos factores oculares en la disminución de los potenciales evocados corticales por estimulación luminosa iterativa.

Los factores oculares considerados por nosotros, que controlan la intensidad luminosa que llega a la retina, fueron: pupila, párpados y nictitante.

La secuencia experimental fué la siguiente:

1. Se estudió la habituación en gatos con ojos normales. Los potenciales comienzan a disminuir a las 2 horas, desapareciendo a las 4 horas.

2. Los mismos animales fueron registrados previa instilación de atropina 1 % en ambos

ojos. Se obtuvo disminución del potencial que llega a un máximo de 15 % a las 10 horas.

3. Se paralizaron los párpados, extirparon las nictitantes y se instiló atropina. En estas condiciones los potenciales fóticos corticales no disminuyeron aún después de 24 horas de estimulación.

Estos resultados señalan la importancia de los factores oculares en el fenómeno descrito como habituación fótica cortical.

Retardo de la habituación y facilitación de los potenciales evocados fóticos en cuerpo geniculado lateral del gato por efecto de la sección pretrigeminal. A. ROJAS L. DE GUEVARA, W. LIFSCHITZ, A. PÉREZ Y N. FERNÁNDEZ. *(Inst. Fisiología y Cát. Fisiopatología, Univ. Chile, Santiago, Chile).*

En gatos normales crónicamente implantados se estudió con técnica oscilográfica la habituación de las respuestas evocadas fóticas en corteza visual y cuerpo geniculado lateral. La pupila fué previamente dilatada mediante la instilación conjuntival de atropina 1/100 y la distancia de la cabeza del animal al foco del fotoestimulador mantenida constante. Una vez estudiada la habituación se realizaron las lesiones pretrigeminales electrolíticamente.

En 27 animales normales controlados, la habituación fué muy discreta e inconstante, y en algunos casos no se observó habituación después de 8 horas de estimulación.

Cuando la habituación se manifestó, se inició en corteza en algunos animales, pero en otros comenzó a nivel del cuerpo geniculado o marchó paralelamente.

Tanto las secciones pretrigeminales mediopontinas como las rostropontinas provocaron aumento de magnitud del potencial evocado y retardo en la habituación de las respuestas a nivel del cuerpo geniculado lateral, es decir efectos similares a los descritos por otros autores en corteza visual. En dos casos se observó facilitación del potencial evocado en geniculado lateral sin modificaciones de la respuesta en corteza. Los componentes tardíos del potencial evocado fueron los que experimentaron un mayor aumento.

New technique for the labyrinthectomy in batrachians. J. SIMÕES, JR. *(Depto. Fisiología, Esc. de Odontología, Univ. de Bahía, Salvador, Bahía, Brasil).*

Most of the experimental programs of Physiology have, at least, a point to show the participation of the labyrinth in the maintenance of the muscular tonus, therefore, in the postural regulation.

Several techniques have been employed but all of them have proved to be very difficult to perform and, in general, the way the animal is handled gives the audience a feeling of extreme cruelty.

Trying to solve this problem the author conceived a type of mouth opener for batrachians which made it easy to reach the labyrinth through the mouth, without the crudeness of former techniques. The results, however, were not entirely satisfactory and he presented as an alternative technique to destroy the labyrinth the use of a single steel stylet (a strong, sharp wire would do).

The experiments were done with adult frogs *(Leptodactylus Pentactylus)* and consisted in the perforation of the tympanic membrane followed by introduction of the stylet in the wall of the mastoid until it reached the labyrinth. It was easily destroyed by a circular motion impressed on the stylet.

Confirmation of the labyrinthetic lesion is given by a statical attitude (extension of the limbs of the opposite side) and the type of locomotion observed (in circles).

The author has obtained satisfactory results with this simple, rapid and safe technique.

Estudios electrofisiológicos sobre la interacción complementaria entre la neurona y la célula glial. G. SVAETICHIN, G. MITARI, E. VALLECALLE, J. VILLEGAS Y M. LAUFER. *(Lab. Fisiología, Inst. Venezolano Investigaciones Científicas, Caracas, Venezuela).*

En la retina del pez se demuestra el origen intracelular del potencial-S, registrándose el tipo-L de respuesta (luminosidad) en las células horizontales y el tipo-C (cromático) en las fibras de Müller, espacio glial extraneuronal de las células bipolares; registros correspon-

dientes se obtuvieron a nivel de las dendritas proximales de las células ganglionares. Son considerados tres sistemas funcionales: 1) Células horizontales externas, reflejan solamente la actividad de los conos; 2) Células horizontales internas, en relación con conos y bastones; 3) Fibras de Müller, unidad analítica con bipolares predominantemente operadas por conos.

Observaciones hechas en distintas condiciones de adaptación, oxigenación y temperatura mostraron que el umbral y la amplitud del potencial-S son inversamente proporcionales a la magnitud del potencial glial de membrana (GMP), para la respuesta-L la fase de hiperpolarización de la respuesta-C, y directamente proporcional (dentro de ciertos límites) para la fase de depolarización de la respuesta-C.

Se concluye que la adaptación retineana depende principalmente de fenómenos neurofisiológicos determinados por la interacción bipolar glial y que el GMP depende de la actividad y de las concentraciones iónicas de ambas células, comportándose funcionalmente la doble membrana que las separa como una sola y formando ambos elementos celulares un comportamiento iónico donde la glia actúa como puente entre la neurona y la corriente sanguínea.

Cerebello-reticular interaction on spinal reflexes. C. TIMO-IARIA AND J. ANTUNES-RODRIGUES. *(Depto. Fisiología, Fac. Medicina Ribeirão Prêto, São Paulo, Brasil).*

In order to investigate some interactions between cerebellum and the mesencephalic reticular formation the following experiments were performed: group Ia afferents of either the *gastrocnemius* or *tibialis* nerves of adult cats were stimulated by single pulses of 2 msec duration and voltage just enough to evoke 50 % of the maximal monosynaptic potential, recorded from L_7 ventral root; previous stimulation of the ipsilateral reticular formation (single pulses of 5 msec, 8-10 V) at definite intervals between 0 and 200 msec, induces striking changes of the excitability of the monosynaptic arc; such changes follow a polyphasic and complex time course.

After extirpation of some cerebellar areas such effects are significantly modified. Destruction of the ipsilateral anterior lobe usually enhances the late facilitation; ablation of the ipsilateral paramedian lobe strongly depresses such facilitation and destruction of the whole posterior lobe has similar effects on the excitability curves.

It seems that a cerebellar component is present in the mesencephalic reticular influences on the investigated spinal reflexes; such component is detected within the 30-50 msec range in a great number of experiments.

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Propiedades de la respuesta de las células gliales en relación a la actividad de las células ganglionares de la retina. J. VILLEGAS. *(Dept. Fisiología, Instituto de Investigaciones Científicas, Caracas, Venezuela).*

Se han estudiado los patrones de respuesta de células ganglionares aisladas en la retina del pez (*Centropomidae*), relacionándolos con las respuestas de las células gliales.

La respuesta glial (potencial-S), aún no siendo considerada como potencial generador de la descarga de las células ganglionares, presentó una amplitud "umbral" (1.2 mV), siendo ésta la amplitud de la reacción glial provocada por los procesos neuronales que inician el disparo de las células ganglionares.

Los campos receptivos de las células ganglionares aisladas mostraron un umbral constante en toda su extensión para cada unidad, no presentando diferencias en el patrón de respuesta entre el centro y la zona periférica cuando fueron explorados mediante un estímulo luminoso, blanco o monocromático, de 25μ de diámetro. Se observó el cambio del patrón de respuesta de una célula ganglionar tipo "on" al tipo "off", provocado por la adaptación del punto de registro y estimulación a un nivel más alto de luminosidad, siendo explicado este fenómeno en función de los cambios que experimentan el potencial de reposo y la respuesta de las células gliales durante los procesos de adaptación. Este hecho, así como la concordancia entre la magnitud del plateau de la curva de distribución espacial de amplitud de la respuesta glial y los diámetros de los campos receptivos de las

células ganglionares, son interpretados como evidencia de la existencia de unidades funcionales glia-bipolares conectadas a una célula ganglionar y parcialmente relacionadas con otras células ganglionares.

Se describen células ganglionares cuya sensibilidad espectral es idéntica, pero presentan patrones de respuesta opuestos en su relación temporal con la estimulación. Los puntos máximos y de inflexión de la curva de respuestas espectrales de los distintos tipos de elementos ganglionares coincidieron con los puntos correspondientes de la curva de respuestas espectrales de los distintos tipos de células gliales. Dos grupos diferentes de célula bipolar son asumidos; el primero sería eficaz para la excitación de las células ganglionares durante la fase de hiperpolarización relativa de la respuesta glial, en tanto que el segundo lo sería durante la fase de depolarización relativa de la respuesta glial. Estos dos tipos de célula bipolar explicarían la existencia de tipos opuestos de patrón de respuesta de las células ganglionares. Se considera que estos hechos reflejan los mecanismos retinianos para el contraste espacial (blanco-negro, colores oponentes).

Se describen registros intracelulares de los potenciales dendríticos y del cuerpo de las células ganglionares.

PATOLOGÍA GENERAL
GENERAL PATHOLOGY

Injeções periódicas de sangue em aves. Implicações genéticas. A. BUSCHINELLI. *(Depto. Biologia Geral, Fac. Filosofia, Rio Claro, SP, Brasil).*

Typification of *Staphylococcus aureus* by bacteriophages and sensitivity tests to antibiotics. R. DARRICARRERE-TORBALAY AND R. HERRERA LOBOS. *(Depto. Microbiología. Esc. Medicina, Univ. Concepción, Chile).*

Staphylococcal infections represent, because of its epidemiological features, one of the most serious problems of Medicine, both in its therapeutic and preventive aspects. This fact is closely related to the gradual increasing resistance of these microorganisms to antibiotics, which has been observed since introduction of these drugs in therapeutic.

The purposes of this paper have been to determine, by means of bacteriophages, the types of staphylococci prevailing in our hospital environment and its sensitivity to antibiotics.

Bacteriophage typing and sensitivity antibiotic tests of 517 strains isolated from different patients showed the following results: 37.2 % were not tipifiable. In 62.8 % of tipifiable strains was observed that the most prevalent were types 80 (16.8 %), 80/81 (16.2 %) and 81 (7.7 %).

The study of susceptibility to antibiotics revealed a high rate of resistant strains, particularly to Penicillin, Streptomycin and Oxitetracycline. This resistance was higher among the corresponding strains to the types mentioned above and lower for the I, II, III groups of Phages.

This paper shows, besides, that staphylococci offer a high resistance, though less striking than that showed to antibiotics, to antibacterial drugs recently introduced in our environment, and it demonstrates that the most effective in staphylococcal infections treatment would be erythromycine, sigmamycin and novobiocin. Results obtained in this work show that some phage-susceptible staphylococci present a higher antibiotic resistance and that these strains correspond to those which appear more frequently associated to staphylococcal infections ocurring in hospital environment.

The reservoir of these strains is the nasopharynx tissue, in the skin and clothes of hospital personnel as nurses, auxiliaries, etc., or patients with clinical or inapparent infections. These facts and the circumstance that the strains are propagated by direct or indirect contact through contaminated clothes or tools or by the air, permit to conclude that the prevention of staphylococcal infections must reside principally in the adoption of measures tending to get a better asepsis and hospital hygiene and to increase organic resistance of individuals to depend less in the use of new antibiotics.

Bacteriuria. R. DARRICARRERE-TORBALAY, E. VIVALDI & R. ZEMELMAN. *(Deptos. Microbiología y Fisiopatolo-*

gía, Esc. Medicina, Univ. Concepción, Chile).

Bacterial multiplication in the urinary tract represents a pathological fact. This circumstance has been denominated "True Bacteriuria" to distinguish it from the "urinary contamination", which is the consequence of the entrance of bacteria in the urine during the recollection of the specimens.

The fundamental difference of these circumstances resides in the number of microorganisms per milliliter of urine. In the urinary infection, the number of bacteria is high, being generally over 100 000. However in the urinary contamination, bacteria number per milliliter of urine is always under 10 000 and generally, around 100 and 1 000 per milliliter of urine.

The importance of the infection of the urinary tract in children and the difficulties arisen for its diagnosis, has moved us to study a method which permits to evidence the asymptomatic infections and contribute to a better knowledge of its evolution and prognosis.

For this purpose, appropriate medium culture were sewed with increasing dilutions of urine taken from 90 children and collected aseptically.

85.7 % of the children, in serial examinations, presented less than 1 000 bacteria per ml of urine, 9.9 % showed quantities inferior to 9 000 and only 4.4 % revealed counts higher than 10 000 bacteria per ml of urine. That is, only in this last group of children asymptomatic true bacteriuria was verified. In these cases, bacterial species of the Coliformis and Proteus group were identified and in those of contamination, specially staphylococcus albus and coliformis.

The clinical implication of these findings is difficult to evaluate; but, it is undoubtless, that at the same time that it demonstrates that true bacteriuria represents a pathological fact, they claim for a close relationship between the clinic and the laboratory, in order to adopt more rational measures in the prevention and control of urinary infection.

Bacteriuria persistente y acidificación urinaria. C. DELLUCCHI, E. VIVALDI, M. BELMAR Y R. ZEMELMAN. *(Deptos. Fisiopatología y Bacteriología, Univ. Concepción, Concepción, Chile).*

La infección urinaria crónica constituye un cuadro frecuente de observar en clínica, siendo su tratamiento difícil, ya que la curación sólo se alcanza en un 10 % a 20 % de los casos. La bacteriuria persistente puede inducir infección aguda del tracto urinario y ser la responsable de la mantención de la actividad en los procesos crónicos. La acidificación de la orina por ingestión diaria de Metionina impide o dificulta la multiplicación bacteriana.

Se someten a estudio 9 pacientes de ambos sexos, de edad variable, que sufrían de infección urinaria crónica no obstructiva, de varios años de evolución. Todos ellos habían sido sometidos previamente, y en forma repetida a tratamiento con diversos antibióticos y otras medidas terapéuticas. El control del recuento bacteriano en la orina fué superior, en todos, a 100 000 gérmenes por ml. La administración de Metionina, en cantidad suficiente para bajar el pH urinario a valores de alrededor de 5, determinó un descenso del recuento bacteriano entre el día 15 y 20. La continuación de la administración de la droga permitió la esterilización de la orina a partir del segundo mes, concordando con la mejoría clínica y del sedimento urinario.

Pielonefritis experimental ascendente. E. VIVALDI, C. DELLUCCHI, M. BELMAR Y R. ZEMELMAN. *(Deptos. Fisiopatología y Bacteriología, Univ. Concepción, Concepción, Chile).*

Se ha demostrado la importancia de la bacteriuria persistente en la patogénesis de las infecciones urinarias lo que nos ha llevado a inducir bacteriuria experimental en ratas y perros.

Aproximadamente el 70 % de nuestros animales presentaron signos bacteriológicos y anatomopatológicos de pielonefritis. Estos resultados fueron similares para el grupo sacrificado entre el cuarto y el trigésimo día y aquellos en los que se efectuó la autopsia después de un período de cinco a siete meses. La incidencia de infección renal alcanzó cifras cercanas al 100 % cuando se introdujo en la vejiga, conjuntamente con los gérmenes, una perla

de vidrio que impedía el vacinamiento vesical total. La sección de un uréter, previa a la inducción de bacteriuria, nos demostró que la infección era ascendente, ya que el riñón con uréter seccionado se infectó solamente en los animales con hemocultivo positivo, debido a contaminación peritoneal durante el desarrollo de la técnica experimental.

Se concluye además los resultados de cambios de la presión arterial sistólica en un grupo de nuestros animales.

PSICO-FISIOLOGÍA
PSYCHO PHYSIOLOGY

Aprendizaje de respuestas condicionadas cardíacas con predominio bradicárdico, en ratas neodecorticadas. S. BLOCH, A. TORO Y T. PINTO-HAMUY. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

Ha sido demostrado en este Instituto que ratas neodecorticadas son incapaces de adquirir una respuesta condicionada de evitación ante un estímulo luminoso. Si bien la integridad cortical aparece como esencial para evidenciar un aprendizaje que requiere una respuesta motora compleja, podría no serlo para la expresión de su componente neurovegetativo, como es por ejemplo, la modificación de la frecuencia cardíaca.

Se entrenaron 6 ratas normales y 6 neodecorticadas en una cámara de condicionamiento, empleando una luz como EC y un choque eléctrico como EI. El EKG fué registrado en un polígrafo, mediante electrodos bipolares de plata, insertados en los músculos del dorso.

Los resultados obtenidos muestran que:

1) Tanto las ratas normales como las neodecorticadas presentaron claras evidencias de condicionamiento cardíaco (RCC).
2) Las ratas neodecorticadas tuvieron un porcentaje más elevado de RCC que las normales.
3) Las RCC se expresaron en un aumento o en una disminución de la frecuencia cardíaca, en ambos grupos. La respuesta incondicionada ante el choque eléctrico, en cambio, fué siempre de taquicardia.
4) Las ratas decorticadas tendieron a dar un número mayor de RCC bradicárdicas; las normales, en cambio, presentaron más taquicardias. Esta diferencia se fué haciendo más marcada a medida que transcurrieron las sesiones.

Los resultados sugieren que la corteza cerebral no es esencial para que la rata "interprete" adecuadamente el significado de la luz.

Es interesante destacar que en ausencia de la neocorteza, las RCC tienden a hacerse opuestas a las respuestas incondicionadas. Este predominio de la bradicardia como RC en ratas decorticadas mostraría otro aspecto interesante del rol regulador que juega la neocorteza, sobre los procesos neurovegetativos.

Uropepsinogen and emotional stress. B. CANNATA, J. CASTRO, B. ÁLVAREZ, A. ALONSO Y J. R. E. SUÁREZ. *(Depto. Fisiología, Fac. Medicina, Univ. Nac. de Cuyo, Mendoza, Argentina).*

The psychic participation on gastric secretion being already known, we tried to study the influence of emotional stress on the secretion and excretion of uropepsinogen.

For this we took a group of students of the different courses of this Medical School who had, apparently, no gastric disorders.

In all these cases uropepsinogen determinations were carried out during the students' period of examinations and several days after the end of that period.

The Anson and Mirsky method of hemoglobin digestion and tyrosine determination was used, according to Follin-Ciocalteu's technique.

The results were statistically compared with a standard group.

A statistically significant increase was found in these determinations, carried out during the psychic stress, both in comparison with the standard group and with the same individuals after their period of examinations.

Effect of sexual hormones on a conditioned avoidance response of rats. MARÍA C. LICO. *(Depto. Fisiología, Fac.*

Medicina, Ribeirão Prêto, São Paulo, Brasil).

Sexual hormones are known to alter the electroshock seizure threshold in rats. The question was raised whether those hormones would influence also the acquisition and retention of an avoidance response. Sixty white rats were divided in the following groups of 10 animals each:

1) Males daily treated with 500 μg of testosterone propionate, subcutaneously injected from eve of beginning to just the end of 10 days of training. Since then, injections were administered with intervals of 36 hours.

2) Females treated with 50 μg of estradiol benzoate injected following the same schedule that the previous group.

3) Castrated males.

4) Castrated females.

5) Control males.

6) Control females.

All groups were given 100 training trials during 10 days of 10 trials each. Mean percentage of avoidances per group was considered mean of acquisition.

The conditioned stimulus was a light presented 5 seconds before the unconditioned stimulus (5 seconds shock).

After 100 trials, training was stopped and retention tested at intervals of 15 days, during 3 months. The results show differences statistically significant ($P = 1\ \%$) in acquisition by testosterone-treated and both groups of castrated animals.

Surprisingly higher degree of efficiency was observed in all animals at the first retention test compared with final performance of the training period (Kamin effect?).

Extintion of the conditioned response was early among groups of both castrated and androgen-treated rats. These experiments suggest an evident action of sexual hormones on central mechanisms controlling learning.

According with performances of hormone-treated rats, androgens would evoke inhibition and estrogens facilitation of learning. But the lower performances of both castrated groups points out to a more complex interrelationship.

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Factores psicológicos que influyen en el efecto de una neodecorticación. J. Rossat y A. Stevenson. *(Inst. Fisiología, Univ. Chile, Santiago, Chile).*

Investigaciones previas realizadas en este Instituto en ratas albinas, han demostrado la importancia de ciertos factores psicológicos en el aprendizaje de Respuestas Condicionadas (RC) tanto en ratas normales como con lesiones de la neocorteza cerebral. a) Se demostró que un grupo de ratas seleccionadas por una mayor exploratividad aprenden más rápido que un grupo de animales tomados al azar. b) La experiencia preoperatoria en nuestra situación de condicionamiento instrumental determinaba un más alto rendimiento en el reaprendizaje post-operatorio.

Se quiso ver si el sobreentrenamiento preoperatorio influiría positivamente en el aprendizaje de una RC visual en ratas neodecorticadas.

Se tomaron 2 grupos de ratas y se entrenaron durante 22 sesiones en una RCV instrumental de evitación. El grupo A, experimental (N=13), se sobreentrenó (tomándose como criterio de sobreentrenamiento, el doble de sesiones de entrenamiento). El grupo B, control (N=13), se entrenó 22 sesiones. Ambos grupos fueron neodecorticados. El período de aprendizaje post-operatorio fué de 25 sesiones en ambos grupos.

Se encontró una diferencia significativa entre ambos grupos, comparando los porcentajes de RC de cada sesión a lo largo de 25 sesiones ($U=219$, $z=1.8$; p. .035); esta diferencia no es evidenciada al comparar RC totales entre las ratas de ambos grupos. Se interpretan estos resultados en el sentido que el sobreentrenamiento determinaría un cambio plástico más marcado y estable que facilitaría el reaprendizaje después de una neodecorticación.

Efecto de lesiones extensas en la neocorteza y corteza específica de la rata albina, en el aprendizaje de una respuesta condicionada instrumental auditiva. M. A. Saavedra, N. Clarke y C. Oberti. *(Inst. Fisiología, Esc. Medicina, Univ. Chile, Santiago, Chile).*

La presente investigación se realizó con el objeto de precisar la función del área corti

cal auditiva de ratas albinas en la adquisición de una RCIA. Estudios previos indicaban que, ratas con 65 % de neocorteza lesionada y con compromiso del 79 % del área auditiva, demostraban relativa incapacidad para adquirir este aprendizaje. Nos planteamos la hipótesis del grado en que esta incapacidad se debía a la lesión del área específica y/o al resto de la corteza.

Se tomaron 16 ratas albinas, de las cuales 8 se neodecorticaron dejando la corteza auditiva intacta. Las ocho restantes fueron sometidas a neodecorticación de la zona auditiva exclusivamente. Luego fueron entrenadas en una RC de escape a un estímulo auditivo (tono de 2.500 c/seg y 2 seg duración). El estímulo incondicionado era una descarga eléctrica a través de una parrilla, que el animal evitaba al escapar al compartimiento vecino.

1. El grupo de ratas con lesiones extensas sin compromiso auditivo presentó un déficit significativamente mayor ($U=10$, $p < .02$) que el grupo con lesiones auditivas.

2. La comparación de los dos grupos con lesiones extensas de la neocorteza indican que el compromiso del área auditiva no determina diferencias significativas entre ellos.

3. Se observaron diferencias significativas ($U=20$, $p < .025$) entre el grupo de ratas normales y el grupo que presentaba sólo lesiones auditivas.

Podemos deducir entonces, que la corteza auditiva tiene una función integradora del aprendizaje en general, pero no específicamente de un aprendizaje auditivo de este tipo; es decir, se obtendría igual déficit lesionando un porcentaje semejante de corteza específica e inespecífica.

Diferencia funcional de la neocorteza anterior y posterior de la rata en el aprendizaje de una respuesta condicionada visual. M. A. SAAVEDRA Y T. H. PINTO-HAMUY. *(Inst. Fisiología, Esc. Medicina, Univ. Chile, Santiago, Chile).*

Investigaciones previas relacionadas con la función de la neocorteza de ratas albinas en el aprendizaje de una RCV instrumental, demuestran la importancia de la neocorteza anterior y posterior en la adquisición de este hábito. Un grupo de ratas con lesiones posteriores fué significativamente inferior a un grupo anterior, lo que se atribuyó al compromiso del área visual que tendría un rol funcional específico respecto al estímulo condicionado luz.

Nos preguntamos si en ratas con entrenamiento preoperatorio, la corteza posterior tendría también mayor importancia funcional que la corteza de la mitad anterior.

Después de entrenar 17 ratas en una RCV instrumental de escape, fueron neodecorticadas; nueve constituyeron el grupo posterior y ocho el grupo anterior. Las curvas de reaprendizaje de estos dos grupos se compararon entre sí y con las curvas de aprendizaje del experimento anterior.

1. El grupo posterior ($M=96$), a diferencia de dicho experimento, resultó significativamente superior al grupo anterior ($M=48$) ($U=12$, $p < .005$).

2. Este grupo anterior con experiencia no demostró diferencias con el grupo anterior sin experiencia ($M=53$).

3. El grupo posterior con experiencia ($M=96$), fué significativamente superior al grupo posterior sin experiencia ($M=27$) ($U=9$, $p < .001$).

Los resultados llevan a concluir que existe una diferencia funcional de la neocorteza anterior y posterior: la neocorteza anterior es importante, tanto en la adquisición como en la retención y readquisición de nuestra situación de aprendizaje, y en cambio la neocorteza posterior —esencial en el proceso de adquisición— pierde su importancia una vez que el hábito ha sido establecido. La experiencia previa no influiría en el grupo anterior, siendo por el contrario el factor decisivo para el nivel de rendimiento alcanzado por el grupo posterior.

Efecto de las ablaciones del *gyrus compositus anterior* en reflejos condicionados e incondicionados en perros. H. G. SANTIBÁÑEZ Y B. ZERNICKI. *(Inst. Fisiología y Psicología, Univ. Chile e Inst. Biología Nencki, Acad. Ciencias Polonia).*

En 14 perros fué estudiado el efecto de la extirpación del *gyrus compositus anterior* y zonas adyacentes sobre reflejos condicionados e incondicionados salivares.

Esta ablación fué practicada en etapas su-

cesivas, primero en un hemisferio y luego en el otro.

Después de una operación, homolateral con respecto al conducto salival controlado, se observó disminución de las respuestas tanto condicionada como incondicionada, en una primera etapa y luego en una segunda etapa hay una franca potenciación de ambos tipos de respuestas reflejas.

Después de la segunda operación los reflejos condicionados al comienzo están deprimidos y más tarde se hacen hipernormales.

El decrecimiento antes señalado, dura algunas semanas mientras que la potenciación subsecuente dura desde varias semanas hasta algunos meses.

Después de las operaciones, sean unilaterales o bilaterales hay una pérdida de los reflejos inhibitorios por algunos días.

La alteración de los reflejos defensivos producidos por ácido son mayores que la observada en los reflejos alimenticios.

Utilizando el método de referencia se observa que los umbrales están incrementados.

SANGRE
BLOOD

Método para el estudio continuo de la concentración de oxígeno plasmático "in vivo". L. A. ESCARCENA. *(Serv. Fisiología Obstétrica, Fac. Medicina, Montevideo, Uruguay).*

Estudio con Fe-59 del efecto eritropoyético del plasma de ratas irradiadas y anemizadas con fenilhidracina. I. ESKUCHE. *(Santiago, Chile).*

Ratas expuestas a 550 r de rayos gama (Cs) y luego tratadas con fenilhidracina, desarrollan una anemia intensa (< 3 g Hb/100 ml) que dura aproximadamente dos semanas. Durante el período de anemia máxima, el plasma de estas ratas contiene tasas altas de eritropoyetina, mayor que 20 unidades cobalto por ml. El efecto eritropoyético es una función lineal del grado de anemia para valores de hemoglobina menores que 7 g/100 ml. Ratas solamente irradiadas desarrollan una anemia leve (Hb < 9 g/100 ml) y el plasma de estos animales no muestra actividad eritropoyética.

Un estudio del efecto del plasma de ratas irradiadas, con anemia hemolítica, sobre la distribución del ^{59}Fe en ratas ayunadas, medido a diferentes intervalos de tiempo después de la inyección del plasma, mostró una respuesta bifásica con máximos a las 18 y 48 horas. Este hallazgo sugiere la existencia en este plasma de por lo menos dos factores que actúan sobre los elementos eritroides ya existentes, aumentando la síntesis de hemoglobina —aumento precoz de la incorporación de ^{59}Fe a los eritrocitos— y otro que actuaría estimulando la diferenciación de elementos precursores a células eritroides — efecto característico de la eritropoyetina que se manifiesta por un máximo de incorporación de ^{59}Fe en los eritocitos a las 48 horas después de la inyección del plasma.

Influencia del calcio, eritrocitos, temperatura y alimentación en la estabilidad plaquetaria. R. C. HONORATO Y R. T. VÁSQUEZ. *(Lab. Química y Química Fisiológica, Esc. Dental, Univ. Chile, Santiago, Chile).*

1. Las plaquetas humanas que son inestables cuando van acompañadas de eritrocitos, se estabilizan al ser estudiadas en plasma nativo.

2. Las plaquetas estudiadas en sangre total con citrato de sodio 0.2M (1p. para 9p. de sangre) son estables.

3. La estabilidad de las plaquetas estudiadas en sangre total, hecha incoagulable con oxalato de sodio 0.025M o con oxalato de sodio 0.1M se presenta alterada, a pesar de haberse eliminado el calcio.

4. La eliminación de los eritrocitos de la sangre obtenida con oxalato de sodio 0.025M se traduce en estabilización de las plaquetas.

5. Las temperaturas muy bajas, disminuyen la estabilidad de las plaquetas, aunque se hayan eliminado los eritrocitos.

6. La ingestión de alimentos en mayor cantidad que una taza de té y un trozo de pan con o sin mantequilla (almuerzo), hace que las plaquetas en plasma nativo sean retenidas en las resinas de intercambio catiónico como si fueran acompañadas de eritrocitos.

Influence of ionic strength on stability of human blood platelets. R. HONORATO, G. MAJLIS AND R. VÁSQUEZ. *(Labor. Química y Bioquímica, Escuela Dental, Universidad Chile, Santiago, Chile).*

The number of human blood platelets destroyed or fixed by glass contact are greatly increased using as anticoagulant 0.025 M sodium or potassium oxalate (1 part to 9 parts of blood).

This alteration is notably diminished by increasing the ionic strength with NaCl or KCl.

Stability of human blood platelets exposed to glass contact clearly improves if ionic strength is increased with NaCl or KCl using no anticoagulant.

However 0.025 M KCl acts as 0.025 M, sodium or potassium oxalate in connection with the number of human blood platelets fixed or destroyed by glass contact.

Hemolytic activity of animal venoms. II. Variation in relation to erythrocyte species *. E. M. A. KELEN, G. ROSENFELD AND F. NUDEL. *(Labor. Hematología, Instituto Butantan, São Paulo, Brasil).*

Sensitivity of human, rhesus, dog, horse, ox, sheep, rabbit, guinea-pig, rat and mouse erythrocytes to hemolysins of some animal venoms (17 snakes, 10 spiders and 2 scorpions) was investigated. Indirect hemolytic activity of venoms was determined on erythrocytes by estimating colorimetrically the liberated hemoglobin.

There was no correlation between venoms hemolytic activity and zoological classification of animals whose erythrocytes were studied.

Snake venoms, in general, hemolyzed all 10 erythrocyte species, with the exceptions of venoms of *Micrurus frontalis* that did not hemolyze human and ox erythrocytes, and of *Bothrops cotiara* that did not hemolyze the same cells besides those of rhesus, sheep and rabbit.

Spider venoms of *Theraphosidae* family hemolyzed only mouse red cells and some of them also the rat cells. The one of *Loxosceles* hemolyzed with intensity human corpuscles and weakly those of ox. *Lycosa* venom lyzed ox and sheep erythrocytes, and weakly so human erythrocytes. The venom of *Phoneutria* was not hemolytic. All of them were inactive to erythrocytes not referred to.

Venoms of *Tityus bahiensis* and *Tityus serrulatus*, the two scorpions most frequently found in Brazil, were not hemolytic to anyone of the erythrocyte species.

The sensitivity degree of each erythrocyte species was calculated to the hemolysis by all venoms, but it was observed that very sensitive erythrocytes could be resistent to certain venoms and vice-versa.

The absence of cholesterol in a serum used as lecithin supplier can activate hemolysis by some venoms, but, on the other hand, it can inhibit activity of others.

There is specificity of erythrocytes sensitivity to each venom, demonstrating the complexity of hemolytic activity that must be the effect of various factors. Therefore it is necessary to indicate the species of the erythrocyte used when defining the hemolytic activity of animal venoms.

(*) This work was aided by a grant of the "Fundo de Pesquizas do Instituto Butantan".

Efecto de un complejo lipídico cerebral en la generación de trombina. A. MOENA-GÓMEZ, S. MUÑOZ, R. SÁNCHEZ Y A. RUIZ. *(Depto. Bioquímica, Univ. Concepción, Concepción, Chile).*

Un factor lipídico complejo extraído de cerebro humano, ha sido ensayado en la preparación de trombinas obtenidas con las técnicas de Quick y Mac Farlane, empleando plasma humano y de cordero.

Se hace un estudio comparativo entre ellas y con el agregamiento del complejo lipídico. Las trombinas obtenidas con dichas técnicas, son notablemente activas y se estudia su estabilidad, ya sea mantenidas en la nevera, como también liofilizadas.

Se estudia el efecto que produce el agregamiento del factor lipídico en la actividad trombínica, según sea la etapa en que es agregado el complejo citado.

Stimulant effects of urinary extracts with high erythropoietic activity on the biosynthesis of nucleic acids. M. PERRE-

TTA, LILIANA ROSMARÍN AND MARÍA PIEBER. *(Sec. Biofísica, Inst. de Física, Matemáticas, Univ. Chile, Santiago, Chile).*

Results previously reported have shown that urinary extracts with high erythropoietic activity increase, *in vitro*, the incorporation of C-14 formate into the RNA purines of liver and spleen of normal rabbits.

The same pattern was obtained in experiments *in vitro*, but a difference was found on the incorporation of C-14 formate into the RNA and DNA purines of bone marrow. While *in vivo the* incorporation was increased, no difference was shown *in vitro*.

As the urine extracts contain an erythropoietic factor, erythropoietine, it was postulated that this substance, *in vivo*, may stimulate *de novo* nuclei acids biosynthesis in bone marrow acting indirectly first over liver or spleen.

In this brief report we show some experimental evidences demonstrating that the liver plays an important rôle in the biosynthesis of the nucleic acids of bone marrow enhancing its activity with the action of the erythropoietine.

The mechanism is being studied *in vitro* and *in vivo* using as an estimation of the biosynthesis the incorporation of $P^{32}O_4H^{=}$, C-14 formate and C-14 glycine into the nucleic acids of bone marrow, liver and spleen of normal rabbits and rats.

Absence of activity of estrogen and androgen hormones on the prothrombin complex of dogs treated with dicoumarol (*). G. ROSENFELD E L. NAHAS. *(Labor. de Hematología, Inst. Butantan, São Paulo, Brasil).*

Dogs were treated with 200 mg of dicoumarol in order to diminish the blood concentration of factor VII and prothrombin; they were injected afterwards, with estrogen and androgen hormones. Comparison made with the control group showed that the hormones, when applied on the 5th day did not accelerate the regeneration of the factors affected by dicoumarol nor were they shown to be able of impeding or diminishing the reduction rate of the factores concerned when simultaneously administered with the anticoagulant.

In dogs submitted to a single treatment with dicoumarol, intravenous injections of water-soluble conjugated estrogens prove not to be able of stimulating the production or eventual release of factors of the prothrombin complex within 1, 2, 3, 4 and 24 hours.

(*)This work was aided by a grant of the "Fundo de Pesquizas do Instituto Butantan".

Efectos de la nefrectomía sobre la población y proliferación eritroblástica de la médula ósea de la rata. J. L. SCARO. *(Inst. Biología de la Altura, Univ. Nac. Tucumán, S. S. de Jujuy, Argentina).*

Se estudió el efecto de la nefrectomía bilateral sobre la producción eritroblástica de la médula ósea. Se estudió la población eritroblástica y los valores del índice mitósico en ratas con bloqueo mitósico con colchicina.

La nefrectomía bilateral produce después de 38 horas de haber sido realizada, una desaparición completa de eritroblastos basófilos en la médula ósea.

Asimismo los animales nefrectomizados mostraron una disminución altamente significativa de la proliferación eritroblástica medida con el test statmocinético. La eritropoyesis fué esencialmente normal en ratas con ligadura de ambos uréteres.

Ratas nefrectomizadas y sometidas a una sangría única masiva, a la administración de cobalto o a la hipoxia hipóxica no mostraron aumento en la proliferación eritroblástica observada en animales normales o con ligadura de uréteres sometidos a esos mismos estímulos de la eritropoyesis.

La nefrectomía bilateral impide completamente el aumento de la actividad eritropoyética del plasma que se hace presente en animales normales o con ligaduras de uréteres, cuando se los somete a una hemorragia aguda, hipoxia hipóxica o administración de cobalto.

Las ratas con nefrectomía bilateral retienen la capacidad de responder a la eritropoyetina exógena.

Erythropoietin production by perfusion of the isolated kidney. E. O. ZANGHERI, H. CAMPANA, F. PONCE, J. C. SILVA, F. O. FERNÁNDEZ AND J. R. E.

SUÁREZ. *(Depto. Fisiología, Fac. Medicina, Univ. Nac. Cuyo, Mendoza, Argentina).*

Perfusions on the isolated kidney of dog were performed during three hours with a Shuster-Dale type pump, using total blood to which the oxygen being consumed by the kidney was not replaced.

The erythropoietic activity of the plasma was determined before and after perfusion, by bio-assay in fasting rats using Fe^{59} as a tracer.

The activity of this radioactive isotope was measured in the plasma, erythrocytes and liver of the rats, three hours after having been injected according to the method of Hodgson and col. The time constant of Fe^{59} disappearance from plasma and the plasma iron turnover, was calculated.

The erythropoiesis of the rats injected with the plasma obtained after the perfusion, was significantly greater than in the control groups injected with the aliquot part of the same not perfused plasma.

Erythropoietic action of tissue extracts. E. O. ZANGHERI, F. O. FERNÁNDEZ, H. CAMPANA, F. PONCE, J. C. FASCIOLO AND J. R. E. SUÁREZ. *(Depto. Fisiología, Fac. Medicina, Univ. Nac. de Cuyo, Mendoza, Argentina).*

Pig's kidney cortex extracts were prepared as carried out for the obtention of renin.

This material was injected in fasting rats to determine the erythropoietic activity. In these animals, the 3-hour test with Fe^{59}, of Hodgson and collaborators, was carried out determining the percentage of the tracer dose injected which is found in the plasma, erythrocytes and liver, and also determining the time constant of Fe^{59} disappearance from plasma and the plasma iron turnover.

With respect to the control group who received injections of isotonic saline solution, the renin-type of renal extract significantly increased the erythropoiesis of the rats.

The same extract, subjected to boiling heat in a water bath during ten minutes, at pH 5.5, maintained its erythropoietic activity, having totally lost its renin activity.

Pig's spleen and liver extracts prepared in the same manner as the renal ones (renin type and subjected to heat) were tested, and they were also active in both forms, though the hepatic ones showed less activity than those of the spleen and kidney.

ENSEÑANZA MÉDICA
MEDICAL TEACHING

Algunos aspectos docentes de la farmacología experimental. L. BARDISA. *(Depto. Farmacología, Esc. Medicina, Univ. Concepción, Concepción, Chile).*

Durante muchos años el sistema de complementar las exposiciones teóricas de Farmacología con un programa de Trabajos Prácticos y Demostraciones no satisfacía plenamente a quienes tenemos la responsabilidad de dicho curso. Por este motivo, se ensayó en 1960 complementar este Curso con Seminarios Experimentales de Farmacología en los cuales grupos de cuatro alumnos debían desarrollar temas particulares en varias sesiones de trabajo, estudiar los aspectos bibliográficos, analizar y elaborar los resultados y hacer una presentación pública según las normas convencionales de un congreso científico.

Los resultados y el interés de los alumnos por esta modalidad excedieron nuestras mejores expectativas.

Teaching of bacteriology. R. DARRICARRERE-TORBALAY. *(Depto. Microbiología, Esc. Medicina, Univ. Concepción, Concepción, Chile).*

Microbiology must be taught to students of a School of Medicine, as the study of the biology of infections and of the reciprocal relations of two biological systems: the host and the parasite.

To achieve this objective, teaching of Bacteriology includes, in the University of Concepción, two stages: one, to the level of the Third Year, during the Preclinical Basic Sciences, and the other, in the course of the Clinical Sciences.

During the first stage, an intensive and practical course about Microbiology and gene-

ral immunology and on the pathogenic mechanisms of bacterial and viral infections is imparted. Teaching includes from the pure biological knowledge of bacteria, rickettsiae and virus to the study of the mechanisms developed by the microorganisms to defeat host resistance, as well as those that the host puts into practice to maintain functional integrity.

This teaching is complemented with a series of theoretical and practical discussions about bacteriological diagnosis methods, their efficacy, interpretation and limitations.

In the second stage, during the Clinical cycle, teaching is integrated with the Infectious Diseases Course and with that of the Preventive and Social Department. During this time, students apply the knowledge acquired during the first stage to the study of patients, of their family and environmental hygiene problems.

The course takes 180 hours in total, from which 120 are devoted to the preclinical cycle and 60, to the clinical one.

Investigación y docencia básicas de la medicina. A. C. NACIMIENTO. *(Dept. Physiol., State Univ., New York, N. Y., U.S.A.).*

El desarrollo vigoroso y continuo de las ciencias médicas básicas exige un adiestramiento cada vez más riguroso de quienes se dedican a la investigación y docencia de las mismas.

Aparentemente el procedimiento más generalizado para proporcionar dicho adiestramiento en Latinoamérica es el otorgamiento de becas. Sin embargo, por la índole misma de dichas becas, la preparación alcanzada es insuficiente. Además, no es sistemática ni ordenada.

Como es bien sabido, en los países de mayor madurez y experiencia universitarias la preparación de quienes sienten vocación por la investigación y la enseñanza de dichas ciencias se hace por medio de carreras dedicadas integralmente a cada una de ellas. Dichas carreras culminan con un título universitario, Ph. D. (Philosophiae Doctor), en fisiología, biofísica, etc., que en las universidades de primera línea representa un adiestramiento riguroso, sistemático y metódico.

La creación de dichas carreras en Latinoamérica es necesaria y urgente, si se desea formar un plantel de investigadores y docentes altamente competente y capaz de un trabajo sólido y original.

Enseñanza de la biofísica en las escuelas de medicina. M. A. PATETTA-QUEIROLO. *(Fac. Medicina, Univ. Carabobo, Valencia, Venezuela).*

En este trabajo se discuten los elementos fundamentales que a juicio del autor deben orientar la enseñanza de la Biofísica en las Escuelas de Medicina latinoamericanas.

Se discute el contenido de la materia y los temas fundamentales que han de enseñarse, teniendo siempre presente que el objetivo de esta enseñanza no es formar biofísicos sino médicos prácticos de sólida preparación básica. Se analizan las ventajas de estudiar bioestadística, matemática aplicada a la biología, leyes físicas que permiten interpretar los fenómenos biológicos, acción de los agentes físicos sobre los seres vivos (con especial énfasis en los capítulos de radiobiología) y algunas técnicas físicas que el estudiante de medicina debe conocer para poder estudiar con provecho otras materias (Fisiología, Bioquímica, etc.).

Se analiza la duración del curso, tiempo destinado a prácticas de laboratorio y seminarios, comparando la experiencia adquirida en 12 años de enseñanza en dos universidades latinoamericanas (Montevideo y Carabobo) con la de otros países (Reino Unido, USA, URSS, etc.).

Finalmente se muestran los resultados de un ensayo docente realizado recientemente en la Universidad de Carabobo (Valencia, Venezuela) donde se impartió la misma enseñanza de la biofísica a alumnos de 1º y 2º años de Medicina, con resultados muy diferentes y de significación estadística entre ambos grupos.

Enseñanza de las técnicas histológicas en las cátedras de histología. Significación docente; influencia sobre la investigación; interés médico-práctico. J. M. SOSA. *(Inst. Histología, Fac. Medicina, Los Andes, Mérida, Venezuela).*

Se informa sobre los resultados de la inclusión de la enseñanza práctica individual,

de las técnicas histológicas en los programas de Histología Normal, en las Facultades de Ciencias y de Odontología en Montevideo, Uruguay, y de Medicina en Mérida, Venezuela.

Se discute la importancia que tal enseñanza tiene: a) desde el punto de vista docente, educativo para la formación científica del estudiante; b) como actividad estimulante del interés por la investigación científica original; c) para la actividad práctica profesional del futuro médico, odontólogo o veterinario.

O ensino da Farmacologia na Escola Paulista de Medicina e alguns problemas correlatos. J. R. VALLE. *(Lab. Farmacología e Bioquímica, Esc. Paulista Medicina, São Paulo, Brasil).*

O ensino atual da Farmacologia na Escola Paulista de Medicina é ministrado aos alunos da 2.a série durante o 1.o semestre do ano letivo. Os 80 alunos do corrente ano foram divididos, para os trabalhos práticos individuais, em 4 turmas de 20 estudantes.

O curso compreende aulas teóricas, práticas e teórico-práticas conforme o horário e a divisão de turmas: A (1 a 20), B (21 a 40), C (41 a 60) e D (61 a 80).

O curso teórico, às 2.as-feiras, para todos os alunos, consta de palestras, conferências, exibição de filmes ou projeção de diapositivos e visa os problemas gerais da Farmacodinâmica.

O curso teórico às 3.as e 5.as-feiras consta de comentários e discussões sôbre os resultados obtidos e as observações feitas em aulas práticas e teórico-práticas e, também, de visitas a Laboratórios Farmacêuticos, ao Jardim Botânico e a instituições científicas, p. ex. aos Institutos Biológico e Butantan.

O curso teórico-prático, às 4.as e 6.as-feiras, consta de demonstrações de efeitos de drogas em animais ou no homem, com discussão e comentários, pelo assistente, dos resultados obtidos.

Para o curso prático, os alunos de cada turma são distribuídos em 5 grupos de 4. Com o programa minucioso e as intruções gerais o aluno recebe no comêço do curso, por escrito, a orientação prévia referente a cada assunto de maneira que ao se apresentar em aula, já deve ter compreendido o objetivo e a metodologia do que vai aprender experimentalmente.

O curso semestral de março o julho tem vantagens indiscutíveis: para o aluno há a concentração no estudo intensivo de duas Cadeiras afins — a Fisiologia no período da manhã e a Farmacologia no período da tarde; para o pessoal docente a possibilidade de dedicação integral, de agôsto a março, à pesquisa e à renovação do material de ensino, à revisão e à redistribuição da matéria para o período letivo seguinte. A vantagem primordial dêsse sistema, a nosso vêr, está na maior atenção que o corpo docente pode então dedicar aos estudantes pós-graduados e bolsistas.

Numa discussão sôbre problemas do nosso ensino médico os seguintes pontos merecem ser sublimados:

a) ensino a número limitado de alunos por série, na dependência das instalações, do equipamento e do pessoal disponível. O corpo docente previsto para 60 alunos não poderá conter menos de 1 professor, 1 adjunto, 2 assistentes, 1 auxiliar de ensino e 3 monitores;

b) obrigatoriedade de trabalho dos professôres e assistentes em regime de dedicação exclusiva;

c) instalações e equipamento não só de acôrdo com o programa básico mas visando à melhoria do ensino e realização efetiva da pesquisa;

d) integração de professores, alunos e auxiliares nos objetivos da Cadeira e fiel execução do programa graças ao cumprimento do dever de cada um;

e) finalmente, a compreensão, inclusive governal, de que o ENSINO, sendo exigência inadiável, não pode continuar como letra morta de programas que não são ou não podem ser cumpridos.

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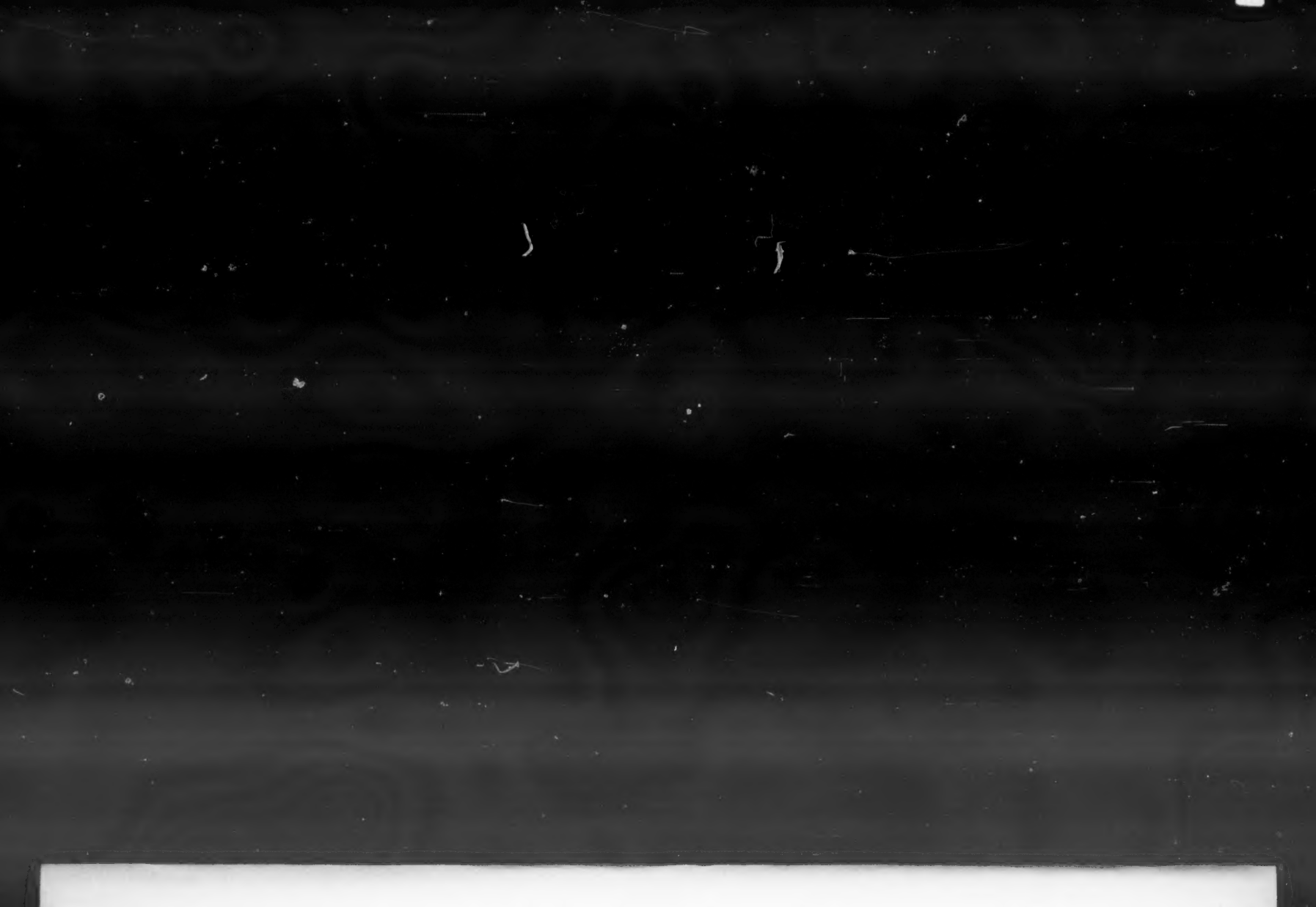

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centímetro	cm	cm	centímetro cúbico	cm^3	cc
milímetro	mm	mm	mililitro	ml	ml
micrón	μ	μ	kilogramo	kg	kg
milimicrón	$m\mu$	$m\mu$	gramo	g	gm
Angström	Å	Å	miligramo	mg	mg
microgramo	μg	μg	miliequivalente	mEq	mEq
hora	h	hr	Curie	c	c
minuto	m	Min	Milicurie	mc	mc
segundo	s	sec	Microcurie	μC	μC
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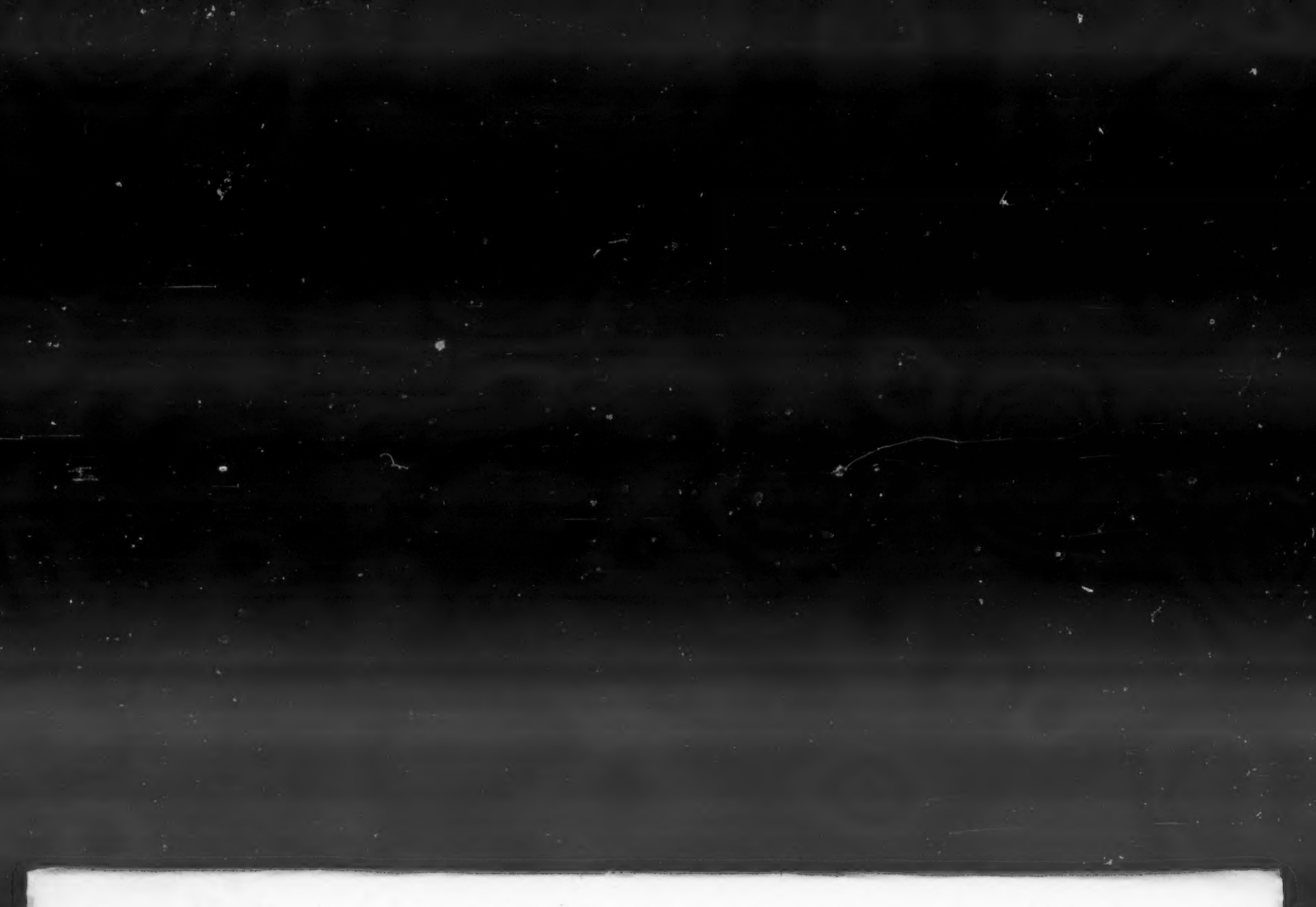
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